The 12th Symposium on Plant Biotechnology
Vol. 12 : 102~111(1998)r

# THE MOLECULAR GENETIC ANALYSIS OF LEAF SENESCENCE

Joon-Hyun PARK, Hye Ryun WOO, Hong Gil NAM

Department of Life Science, Pohang University of Science and Technology, San 31, Hyoja Dong, Nam Gu, Pohang, Kyungbuk, 790-784, Korea

## INTRODUCTION

Programmed cell death in plants is found in many developmental and environmental response processes. These processes include relatively localized cell death, occurring in hypersensitive response and in differentiation of tracheary elements, as well as senescence at an organ or a whole plant level.

Leaf cells experience sequential disorganization of cellular organelles and dramatic changes of cellular metabolism during leaf senescence (1-7). The metabolic changes include loss of photosynthetic activities and hydrolysis of macromolecules built up during the growth phase. The degenerative activities are often concomitant with massive remobilization of the hydrolyzed compounds to the growing parts of plants such as young leaves and developing seeds. Thus, leaf senescence, although deteriorative in nature, is a critical process for fitness of plants and is regarded as an evolutionarily acquired genetic process.

Due to the biological importance and potential for improvement of crop characteristics such as plant productivity and storage life, there have been quite extensive physiological and biochemical studies conducted on plant leaf senescence during the last decade. However, the molecular and genetic analyses of the mechanism of leaf senescence have been actively investigated only recently (8-10).

## Senescence-associated genes.

In the past several years, continuing efforts have been made to identify genes associated with or induced during leaf senescence. Although the nature of many of these senescence-associated genes (SAGs) is still unknown, some of the genes are providing molecular clues to the molecular status of senescing leaf cells.

The spectrum of genes involved in protein turnover such as cysteine protease-like genes (11-13) is being expanded continuously. The role of proteolytic degradation in leaf senescence is illustrated by biochemical identification of a cysteine protease and a serine protease that catalyze the degradation of Rubisco, a major leaf protein undergoing degradation during leaf senescence (14), and by immunological identification of alkaline endopepetidases which increase during leaf senescence (15). The genes in the ubiquitin pathway such as polyubiquitin genes from potato (16) and *Arabidopsis* are also induced during leaf senescence, in consistent with the previous reports (17-19).

Other SAGs involved in hydrolytic activities include RNases such as the bean ribonclease-like PR protein gene, *Ypr10* (20, 21). RNases have been suggested to function in phosphate remobilization during senescence (22, 23). A pumpkin gene for a key glyoxysomal protein, 3-ketoacyl-CoA thiolase (24), that may be involved in remobilization of fatty acids, is

induced during leaf senescence like other glyoxysomal protein genes (25). A gene for cytosolic glutamine synthetase induced during senescence may function to remobilze the N compounds released during senescence (26-28). Others SAGs include a -glucosidase gene (29) and a gene encoding a peptide related to a endoxyloglucan transferase that may function in cell wall.

Many stress-related genes are also induced during senescence. These include metallothionein-like genes (10, 30-32) that may be involved in chelation of metal ions released during cellular degradation. The genes involved in the oxidative stress response are also induced, including the genes for Fe(II)/ascorbate oxidase (29), anionic peroxidase (12), glutathione S-transferase (13,, 33) and a blue copper binding protein (34). Several pathogenesis-related protein genes are induced in senescent leaves (20, 35, 36). The radish *dinl* gene (37) and its *Arabidopsis* counter part, *senl* (38), are induced by dark treatment. These stress-related genes may participate in protecting the cellular integrity required for progression and completion of senescence.

Study of genes down-regulated during leaf senescence may also provide some information on the molecular mechanism of leaf senescence. Senescence may involve down-regulation of not only many metabolic enzymes but also critical components that repress the senescence program. The down-regulated genes identified recently include the genes for ATP sufurylase (13), a photosystem II 10kD polypeptide (36), and a few stromal enzymes (5).

The SAGs may include 1) the genes executing the senescence program such as genes involved in disintegration or remobilization of macromolecules, 2) the genes involved in protecting cell viability for completion of the senescence process, 3) the genes involved in initiation or triggering of leaf senescence, 4) the genes controlling the progression rate of senescence (Some of the genes in category 1, including RNase, protease or ubiquitin pathway genes may also function in this category). Most of the identified genes are in categories 1 and 2.

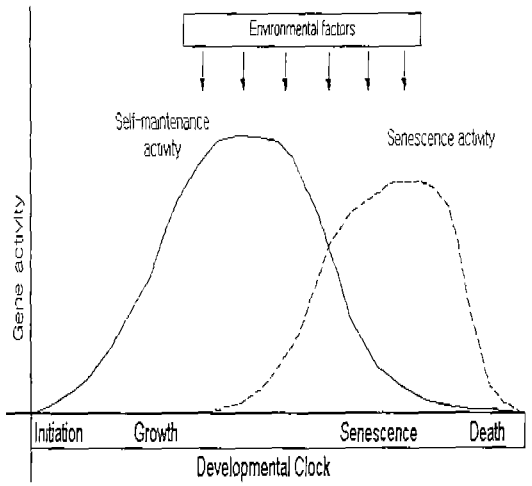


**Fig. 1. A simple model for leaf growth and senescence.**

In this simple model, regulation of senescence is viewed as a matter of balance between two antagonistic self-maintenance and senescence activities. The self-maintenance activity in sum increases during leaf growth and declines at the senescence stage. This activity would include many anabolic activities such as photosynthesis. Other basic cellular maintenance activities will include RNA and protein synthesis and repair activities. The senescence activity may start to accumulate at earlier stages but the resulting symptoms are not apparent until loss of self-maintenance activity at a later stage. In the intermediate stage, the Yin and Yang activities coexist and plant may partly resume the non-senescent stage or proceeds to later senescent stage, depending on the conditions. In this view, the initiation point for senescence can not be clearly defined. Once a certain threshold level of the Yin activity reaches a point of no return, senescence proceeds towards death. The program is basically set by an age-dependent

factors but environmental factors also influence the timing and progression rate of senescence. All these program are supposed to be under genetic control.

## Regulation modes of senescence-associated genes

Analysis of the modes of regulation of SAGs will provide important clues to the molecular mechanism of initiation and progression of leaf senescence. There has been some, although limited, progress in this regards.

Many leaf SAGs are not uniquely induced during the senescence stage. Many genes are shown to be induced in both seed germination and leaf senescence (11, 24, 28). Seed maturation may also have some common gene activity with senescence (35). The *Arabidopsis meri-5* gene (64) and a potato ubiquitin-ribosomal protein gene (17) expressed in leaf meristem are induced during leaf senescence. Some SAGs show a biphasic induction pattern during leaf development, suggesting that they have other roles in leaf development as well as in senescence (8, 13, 36). Some leaf SAGs are expressed also in senescence of other organs or cell types and are not uniquely functioning in leaf senescence.

Many SAGs are regulated by adverse internal and external environmental factors, including heavy metal (30), salicylic acids (20, 35), dark (20, 37, 38), senescence-affecting hormones (ethylene (38), ABA (38), methyl jasmonate (16), wound (16), heat shock (17, 30) and nutrient starvation (22, 23, 32). Thus, many SAGs also function in plants response to these environmental factors. Indirectly, these results may mean that the senescing cells are in a highly stressed cellular states and require expression of these stress-responsive genes to cope with the stressful condition. It is likely that many other stress-related genes are also induced during leaf senescence.

For a few cases, activation of SAGs was observed during leaf senescence induced by environmental factors. Abundance of the tomato SENU mRNAs increases during leaf senescence induced by aging, heat shock or drought (36). The *Arabidopsis senl* gene is activated during leaf senescence induced by age, dark, ABA, or ethylene (38). It will be interesting in these cases to ask whether the genes are responsive specifically to a specific environmental factor that accelerates leaf senescence or they are induced by a presumed senescence signal that is generated by these factors. Identification and examination of cis-acting elements responsive to age or environmental factors should provide some answer to the question. It is likely either one or both mechanisms are operating for regulation of senescence and stress-responsive genes.

As described above, leaf senescence is not conducted by a unique set of senescence-specific genes but mostly utilizes genes which are also involved in other cellular processes. Plant cells might have recruited these genes during the evolution of the leaf senescence program and to have modulated their expression to be senescence-associated in the leaf. However, it is certainly conceivable that leaf cells utilize genes specific to leaf senescence. It will be important to identify such genes to reveal the mechanism of leaf senescence. These genes may include ones that control the basic senescence program, i.e. the age-dependent developmental program.

It is proper to define, although still somewhat vaguely, leaf SAGs at this point. They may be defined as the genes with increased expression during natural leaf senescence (i.e., age-dependent senescence) relative to other developmental stages of a leaf. Leaf senescence can be initiated or modulated by several internal and external factors as well as in an age-dependent manner. Many plant genes responding to environmental factors may not be

associated with leaf senescence and they may be regarded as genes specific to the environmental factors.

One question regarding the mechanism of leaf senescence is whether the molecular states of leaf senescence induced by the many senescence factors are same or not. Some SAGs (36, 38) are induced upon senescence caused by all the conditions examined. However, the modes of regulation of other SAGs suggest that leaf senescence caused by different senescence factors such as aging, dark, ethylene, ABA, or methyl jasmonate involves differential induction of SAGs (64). The result shows that the molecular states of leaf senescence caused by different senescence factors are different (Fig. 2). This situation may mean that the process of leaf senescence may involve a fine tuning mechanism of expression of SAGs in controlling the timing and progression rate of leaf senescence to incorporate complex environmental signals into the senescence program. Leaf senescence, thus, may be envisioned as a complex process where various environmental influences are superimposed on the age-dependent developmental program (Fig. 3). This mechanism should enhance the fitness of plants in ecological settings with ever-changing environments.

Metabolite repression is involved in regulation of SAGs. Sugar starvation induces glyoxysomal genes (24, 25, 39) and the rice metallothionein-like gene (30). The promoter of the *Arabidopsis sen1* gene is activated upon sugar starvation and are repressed by exogenous sugar compounds. Activation of the promoter during dark-induced senescence is closely correlated with reduced level of sugars. Previous studies have shown that sucrose starvation in cultured rice cells caused vacuolar autophagy and reduced cellular viability (40). These results suggest that deficiency of sugar may be one of the factors regulating leaf senescence, although sugar itself may not be a direct signal (9, 25, 39). Sugar- deficiency is a component of various senescence-inducing environments in which photosynthetic activity and resulting sugar production decline, such as shading or adverse conditions.

## Genetic analysis of leaf senescence.

Genetic analysis of leaf senescence has been mostly limited in identifying stay-green varieties from naturally-occurring cultivars (4, 46) and they are continuing subjects of research on the analysis of leaf senescence (47-50). Recent data indicate that the two genetic loci of soybean, *d1* and *d2*, together play an important regulatory role in leaf senescence. The soybean double mutant causes a significant delay in degradation of leaf soluble proteins (47), plasma membrane, and chloroplast (48), although neither *d1* nor *d2* alone delays senescence. The long studied stay-green mutant of meadow fescue (*Festuca pratensis* Huds.), designated Bf993, is unable to carry out oxygenolytic cleavage of the porphyrin-macrocycle, a key step in chlorophyll degradation (50). Recently, utilizing *Arabidopsis* as a model system, four genetic mutants with a delayed senescence phenotype have been identified (64). They fall into three complementation groups. All of the mutations were monogenic and recessive, indicating that they are required for leaf senescence to proceed properly. Several senescence parameters associated with leaf senescence, including both decrease of photosynthetic components (chlorophyll and Rubisco contents, and photosystem II activity) and increase of catabolic activities (RNase and peroxidase), are delayed in the mutants, suggesting that the three genes defined by these mutations are key regulatory elements in leaf senescence. The delayed senescence phenotypes are observed during both age-dependent and dark-induced senescence. Preliminary data suggest that leaf senescence caused by ABA, ethylene, and methyl

jasmonate are also delayed. The genes may function at a common step of senescence caused by these factors. It should be noted, however, that senescence does still occur in these mutants, suggesting there might be a parallel senescence pathway(s) that bypasses action of these genes (Fig. 3).

**Genetic engineering of leaf senescence.**

There has been a considerable interest in breeding a delayed senescence varieties in the hope of increasing crop productivity and the storage life of leaves. For example, delayed senescence with concomitant preservation of photosynthetic apparatus in the *Gd1d2* mutant of soybean increases seed yield by 44% (54). Despite the potential benefits of delayed leaf senescence in agriculture, application of genetic engineering to manipulate leaf senescence has been limited, in contrast to the case of fruit ripening (55). One recent result is particularly exciting and exceeds expectations (56). Cytokinin, a plant hormone, retards leaf senescence in many plants (57). Although, there have been several reports regarding over-production of cytokinin in plants (56, 58), other promoters not specific to senescence caused abnormalities. When the level of cytokinin was autoregulated under the control of a senescence-induced promoter, the transgenic plant exhibited a clear improvement of several traits important in agronomy. The plants showed 50% increase in both seed yield and total biomass. In addition, leaf longevity was greatly enhanced *in planta* or upon detachment. Further tests may be necessary to check if there are no adverse effects of the introduced construct in various environmental conditions. More thorough understanding on the regulation of the promoter and identification of an age-dependent senescence sequence may be required. It is also noted that cytokinin alone may not be sufficient to delay all the symptoms associated with leaf senescence in other plants (38). Reducing the level of ethylene by introduction of antisense ACC oxidase gene (51) under the control of a senescence promoter may be another way to delay senescence. It is notable that in the stay-green mutation of *Festuca*, which retains greenness but not photosynthesis, dry matter production and tillering were lower than in wild type, implying that retention of photosynthetic activity is required for increased crop productivity.

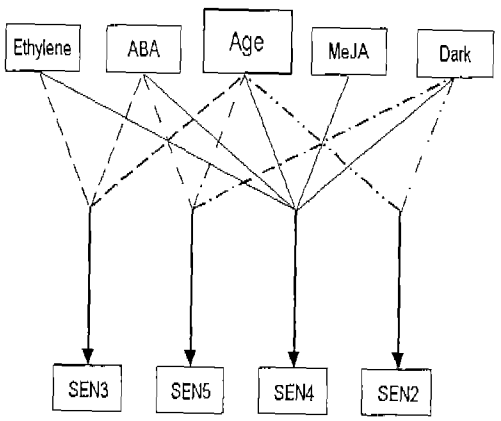


**Fig. 2. Involvement of different sets of genes during leaf senescence affected by various senescence factors.**

Leaf senescence affected by several factors involves induction of different sets of genes. Thus, although the apparent symptoms may look same, the detailed molecular states of senescent leaves are different depending on the senescence factors. For example, the *sen5* gene may be involved in dark-, ABA-, and age-induced senescence but not in ethylene- or methyl jasmonate (MeJA)-induced senescence. In contrast, the *sen4* gene is involved in senescence affected by all the factors. This, in turn, suggests senescence induced different senescence factors may proceed through a common pathway and pathways specific one or more of the senescence factors.

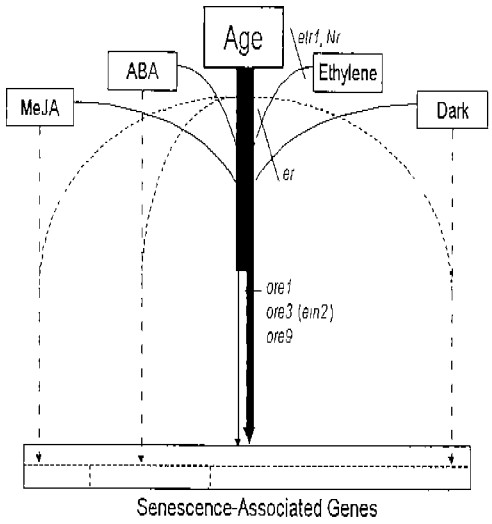


**Fig. 3. Genetic pathways of leaf senescence.**

In this genetic model which is partly hypothetical and partly reflects recent findings, leaf senescence is viewed as a complex process where effects of various environmental factors are superimposed on the age-dependent senescence program. The age-dependent program may be regarded as a basic program. The *Arabidopsis* genes, *ore1*, *3* and *9* defined by genetic mutations lies in a common pathway of leaf senescence. The *er* [63] is in the dark and ethylene pathway but not in the ABA pathway; the effect of the other factors is not known. The *etr-1* gene lies in the ethylene pathway; the effects of the other factors is unknown. Leaf senescence ultimately occurs in all the mutants, suggesting there may be an additional pathway(s) (light line) to senescence; there may be differences in contribution of the pathways to senescence. The hypothetical major pathway is noted by a heavy line. Furthermore, leaf senescence may not go through common pathways; there may be pathways specific to one or more of the senescence factors (dotted lines) and the specific pathways may utilize only a subset of SAGs. The environmental factors may interact before they merge into the age-dependent pathway.

## CONCLUSIONS

The knowledge on the molecular and genetic mechanism of leaf senescence has increased rapidly in the past two years. However, we are still seeing only a glimpse of the molecular activities occurring during leaf senescence and *in vivo* functions of any of SAGs have not been proved experimentally. It will be necessary to identify more SAGs and characterize their function, for example, by transgenic approach. More importantly, we do not know the nature of any key genes that are regulating the initiation or progression rate of leaf senescence, except the *Arabidopsis etr1* gene (59) and the tomato *Nr* gene (53, 59). Isolation of the regulatory genes, specially the genes controlling age-dependent senescence, will be of fundamental importance in understanding the mechanism of leaf senescence. A report on telomere shortening during aging of cultured cells is interesting in this regard (61). Study of the regulation of SAGs is generating some valuable information, but more efforts, including study of transcriptional and posttranscriptional regulation mechanism of SAGs, are certainly needed. In this respect, it is notable that the translational inhibitory activity of ribosome-inactivating proteins increases during leaf senescence (62) and there are reports on the lack of coordination between mRNA and protein levels for some SAGs during senescence (5, 24, 32). Expression of SAGs has been examined mostly at an organ level so far. However, regulation of SAGs may not be same in all cell types of the leaf. The modes of regulation of SAGs at the cellular level need to be examined to further enhance our knowledge on leaf senescence (26).

It is very encouraging that leaf senescence can now be analyzed genetically in *Arabidopsis*, an experimentally amenable model plant. The molecular clones responsible for the mutations should be obtainable with a reasonable effort .

Cloning of these genes along with isolation of more mutants and their characterization should provide critical clues to the mechanism of leaf senescence.

Manipulation of leaf senescence is feasible now through use of a senescence promoter and it contributes significantly to plant improvement. In the future, it should be possible to manipulate leaf senescence using other regulatory senescence genes.


## ACKNOWLEDEGMENTS

The work in the authors laboratory was supported by POSTECH and PMBBRC.


## REFERENCES


1. Nooden LD: The phenomenon of senescence and aging. In *Senescence and aging in plants.* Edited by Nooden LD, Leopold AC. San Diego: Academic Press; 1988:2-50.
2. Smart CM: Gene expression during leaf senescence. *New Phytol* 1994, 126: 419-448.
3. Feller U, Fisher A: Nitrogen metabolism in senescing leaves. *Critical reviews in Plant Science* 1994, 13: 241-273.
4. Nooden LD, Guiamet JJ: Genetic control of senescence and aging in plants. In *The Handbook of the Biology of Aging.* edn4. Edited by Schneider EL, Rowe JW. Orland: Academic Press; 1996: 94-118.
5. Crafts-Brandner SJ, Klein RR, Klein P, Holzer R, Feller U: Coordination of protein and mRNA abundances of stromal enzymes and mRNA abundances of the Clp protease subunits during senescence of *Phaseolus vulgaris* (L.) leaves. *Planta* 1996, 200: 312-318.
6. Matile P, Hoertensteiner S, Thomas H, Kraeutler B: Chlorophyll breakdown in senescent leaves. *Plant Physiol* 1996, 112: 1403-1409.
7. Gay AP, Thomas H: Leaf development in *Lolium temulentum* L: photosynthesis in relation to growth and senescence. *New Phytol* 1995, 130: 159-168.
8. Lohman KN, Gan S, John MC, Amasino RM: Molecular analysis of natural leaf senescence in *Arabidopsis thaliana. Physiol Plant* 1994, 92: 322-328.
9. Hensel LL, Grbic V, Baumgarten DA, Bleecker AB: Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in *Arabidopsis. Plant Cell* 1993, 5: 553-564.
10. Buchanan-Wollaston V: Isolation of cDNA clones for genes that are expressed during leaf senescence in *Brassica napus.* Identification of a gene encoding a senescence-specific metallothionein-like protein. *Plant Physiol* 1994, 105: 839-846.
11. Drake R, John I, Farrell A, Cooper W, Schuch W, Grierson D: Isolation and analysis of cDNAs encoding tomato cysteine proteases expressed during leaf senescence. *Plant Mol Biol* 1996, 30: 755-767.
12. Tournaire C, Kushnir S, Bauw G, Inze D, de la Serve BT, Renaudin J-P: A thiol protease and an anionic peroxidase are induced by lowering cytokinins during callus growth in *Petunia. Plant Physiol* 1996, 111: 159-168.
13. Smart CM, Hosken SE, Thomas H, Greaves JA, Blair BG, Schuch W: The timing of maize leaf senescence and characterisation of senescence-related cDNAs. *Physiol Plant* 1995, 93: 673-682.
14. Yoshida T, Minamikawa T: Successive amino-terminal proteolysis of the large subunit of ribulose 1,5-biphosphate carboxylase/oxygenase by vacuolar enzymes from French bean leaves. *Eur J Biochem* 1996, 238: 317-324.
15. Morris K, Thomas H, Rogers L: Endopeptidases during the developmental and senescence of *Lolium temulentum* leaves. *Phytochemistry* 1996, 41: 377-384

16. Garbarino JE, Oosumi T, Belknap WR: Isolation of a polyubiquitin promoter and itsexpression in transgenic potato plants. *Plant Physiol* 1995, 109: 1371-1378.
17. Genschik P, Durr A, Fleck J: Differential expression of several E2-type ubiquitin carrier protein genes at different developmental stages in *Arabidopsis thaliana* and *Nicotiana sylvestris*. *Mol Gen Genet* 1994, 244: 548-556.
18. Genschik P, Marbach J, Feuerman UM, Plesse B, Fleck J: Structure and promoter activity of a stress and developmentally regulated polyubiquitin-encoding gene of *Nicotiana tabacum*. *Gene* 1994, 148: 195-202.
19. Garbarino JE, Belknap WR: Isolation of a ubiquitin-ribosomal protein gene (*ubi3*) from potato and expression of its promoter in transgenic plants. *Plant Mol Biol* 1994, 24: 119-127.
20. Walter MH, Liu J-W, Wunn J, Hess D: Bean ribonuclease-like pathogenesis-related protein genes (*Ypr10*) display complex patterns of developmental, dark-induced and exogenous-stimulus-dependent expression. *Eur J Biochem* 1996, 239: 281-293.
21. Crowell DN, John ME, Russell D, Amasino RM: Characterization of a stress-induced, developmentally regulated gene family from soybean. *Plant Mol Biol* 1992, 18: 459-466.
22. Taylor CB, Bariolar PA, del Cardayre SB, Raines RT, Green PJ: RNS2: a senescence-associated RNase of *Arabidopsis* that diverged from the S-RNase before speciation. *Proc Natl Acad Sci* 1993, 90: 5118-5122.
23. Bariola PA, Howard CJ, Taylor CB, Verburg MT, Jaglan VD, Green PJ: The *Arabidopsis* ribonuclease gene RNS1 is tightly controlled in response to phosphate limitation. *Plant J* 1994, 6: 673-685.
24. Kato A, Hayashi M, Takeuchi Y, Nishimura M: cDNA cloning and expression of a gene for 3-ketoacyl-CoA thiolase in pumpkin cotyledons. *Plant Mol Biol* 1996, 31: 843-852.
25. Graham IA, Leaver CJ, Smith SM: Induction of malate synthase gene expression in senescent and detached organs of cucumber. *Plant Cell* 1992, 4: 349-357.
26. Sakurai N, Hayakawa T, Nakamura T, Yamaya T: Changes in the cellular localization of cytosolic glutamine synthetase protein in vascular bundles of rice leaves at various stages of development. *Planta* 1996, 200: 306-311.
27. Bernard W, Matile P: Differential expression of glutamine synthetase genes during the senescence of *Arabidopsis thaliana* rosette leaves. *Plant Sci* 1994, 98: 7-14.
28. Watanabe A, Hamada K, Yokoi H, Watanabe A: Biphasic and differential expression of cytosolic glutamine synthetase genes of radish during seed germination and senescence of cotyledons. *Plant Mol Biol* 1994, 26: 1807-1817.
29. Callard D, Axelos M, Mazzolini L: Novel molecular markers for late phases of the growth cycle of *Arabidopsis thaliana* cell-suspension cultures are expressed during organ senescence. *Plant Physiol* 1996, 112: 705-715.
30. Hsieh H-M, Liu W-K, Huang PC: A novel stress-inducible metallothionein-like gene from rice. *Plant Mol Biol* 1995, 28: 381-389.
31. Coupe SA, Taylor JE, Roberts JA: Characterisation of an mRNA encoding a metallothionein-like protein that accumulates during ethylene-promoted abscission of *Sambucus nigra* L. leaflets. *Plana* 1995, 197: 442-447.
32. Thomas H, de Villiers L: Gene expression in rosette leaves of *Arabidopsis thaliana* induced by nutrient deprivation. *J Exp Bot* 1996, In press.
33. Jepson I, Lay VJ, Holt DC, Bright SW, Greenland AJ: Cloning and characterization of maize herbicide safener-induced cDNAs

encoding subunits of glutathione S-transferase isoforms I, II and IV. *Plant Mol Biol* 1994, 26: 1855-1866.

34. Van-Gysel A, Van-Montagu M, Inze DA: Negatively light-regulated gene from *Arabidopsis thaliana* encodes a protein showing high similarity to blue copper-binding proteins. *Gene* 1993, 136: 79-85.
35. Hanfrey C, Fife M, Buchanan-Wollaston V: Leaf senescence in *Brassica napus*: expression of genes encoding pathogenesis-related proteins. *Plant Mol Biol* 1996, 30: 597-609.
36. John I, Hackett R, Cooper W, Drake R, Farrell A, Grierson D: Cloning and characterization of tomato leaf senescence-related cDNAs. *Plant Mol Biol* 1996, In press.
37. Azumi Y, Watanabe A: Evidence for a senescence-associated gene induced by darkness. *Plant Physiol* 1991, 95: 577-583.
38. Oh SA, Lee SY, Chung IK, Lee CH, Nam HG: A senescence-associated gene of *Arabidopsis thaliana* is distinctively regulated during natural and artificially induced leaf senescence. *Plant Mol Biol* 1996, 30: 739-754.
39. Graham IA, Denby KJ, Leaver CJ: Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. *Plant cell* 1994, 6: 761-772.
40. Chen M-H, Liu L-F, Chen Y-R, Wu H-K, Yu S-M. Expression of a-amylases, carbohydrate metabolism, and autophagy in cultured rice cells is coordinately regulated by sugar nutrient. *Plant J* 1994, 6: 625-636.
41. Ye Z-H, Varner JE: Induction of cysteine and serine proteases during xylogenesis in *Zinnia elegans*. *Plant Mol Biol* 1996, 30: 1233-1246.
42. Ye Z-H, Droste DL: Isolation and characterization of cDNAs encoding xylogenesis-sasociated and wounding-induced ribonucleases in *Zinnia elegans*. *Plant Mol Biol* 1996, 30: 697-709.
43. Ryerson DE, Heath MC: Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic stress. *Plant cell* 1996, 8: 393-402.
44. Wang H, Li Juan, Bostock RM, Gilchrist DG: Apoptosis: a functional paradigm for programmed cell death induced by a host-selective phytotoxin and invoked during development. *Plant cell* 1996, 8: 375-391.
45. Mittler R, Lam E: Identification, characterization, and purification of a tobacco endonuclease activity induced upon hypersensitive response cell death. *Plant cell* 1995, 7: 1951-1962.
46. Thomas H, Smart CM: Crops that stay green. *Ann Appl Biol* 1993, 123: 193-219.
47. Guiamet JJ, Giannibelli MC: Nuclear and cytoplasmic stay-green mutations of soybean alter the loss of leaf soluble proteins during senescence. *Physiol Plant* 1996, 96: 655-661.
48. Guiamet JJ, Giannibelli MC: Inhibition of the degradation of chloroplast membranes during senescence in nuclear stay-green mutants of soybean. *Physiol Plant* 1994, 91: 395-402.
49. Canfield MR, Guiamet JJ, Nooden LD: Alteration of soybean development in darkness and light by the stay-green mutation *cytG* and *Gd1d2*. *Ann Bot* 1995, 75: 143-150.
50. Vicentini F, Hoertensteiner S, Schellenberg M, Thomas H, Matile P:Chlorophyll breakdown in senescent leaves: Identification of the biochemical lesion in a stay-green genotype of *Festuca pratensis* Huds. *New Phytol* 1995, 129: 247-252.
51. Grbic V, Bleecker AB: Ethylene regulates the timing of leaf senescence in *Arabidopsis*. *Plant J* 1995, 8: 95-602.
52. John I, Drake R, Farrell A, Cooper W, Lee P, Horton P, Grierson D: Delayed leaf senescence in ethylene-deficient ACC-oxidase antisense tomato plants: molecular and physiological analysis. *Plant J* 1995, 7:

483-490.

53. Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ: An ethylene-inducible component of signal transduction encoded by never-ripe. *Science* 1995, 270: 1807-1809.
54. Guiamet JJ, Terri JA, Nooden LD: Effects of nuclear and cytoplasmic genes altering chlorophyll loss on gas exchange during monorcarpic senescence in soybean. *Plant Cell Physiol* 1990, 31: 1123-1130.
55. Grierson D: Silent genes and everlasting fruits and vegetables. *Nature Biotech* 1996, 14: 828-829.
56. Gan S, Amasino RM: Inhibition of leaf senescence by autoregulated production of cytokinin. *Science* 1995, 270: 1986-1988.
57. Gan S, Amasino RM: Cytokinins in plant senescnece: from spray and pray to clone and play. *BioEssays* 1996, 18: 557-565.
58. Smart CM, Scofield SR, Bevan MW, Dyer T: Delayed leaf senescence in Tobacco plants transformed with *tmr*, a gene for cytokinin production in *Agrobacterium*. *Plant Cell* 1991, 3: 647-656.
59. Schaller GE, Bleecker AB: Ethylene-binding sites generated in yeast expressing *Arabidopsis ETR1* gene. *Science* 1995, 270: 1809-1811.
60. Payton S, Fray RG, Brown S, Grierson D: Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. *Plant Mol Biol* 1996, 31: 1227-1231.
61. Kilian A, Stiff C, Kleinhofs A: Barely telomeres shorten during differentiation but grow in callus culture. *Proc Nat Aca Sci* 1995, 92: 9555-9559.
62. Stirpe F, Barbieri L, Gorini P, Valbonesi P, Bolognesi A, Polito L: Activities associated with the presence of ribosome-inactivating proteins increase in senescent and stressed leaves. *FEBS Lett* 1996, 382: 309-312.
63. Zacarias L, Reid MS: Role of growth regulators in the senescence of *Arabidopsis thaliana* leaves. *Physiol Plant* 1990, 80: 549-554
64. Park JH, Oh SA, Kim YH, Woo HR, Nam HG: differential expression of senescence-associated mRNAs during leaf senescence induced by different senescence-inducing factors in Arabidopsis. *Plant Mol Biol* 1998, 37:45-454