The 5th European Plant Senescence Network Meeting

Wokefield Park, Near Reading, UK

SENnet 2010

SENnet 2010 would like to thank the following organisations for their valuable sponsorship

CONTENTS

The 5th European Plant Senescence Network Meeting

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Scientific and Social Programme

Tuesday 31st August

14.00 onwards	Arrival and registration in the Executive Centre
18.30	Buffet dinner available from Steam Bake Grill in the Executive Centre
	Private bar on the terrace in the Kingfisher Room, Executive Centre
19.30	Welcome (Carol Wagstaff and Tony Stead)
19.40 – 20.40	Welcome and Introduction Private bar and main bar both open

Wednesday 1st September

07.00 – 08.30 Breakfast in Steam Bake Grill in the Executive Centre

(a reception desk will be manned from 08.30 in the Mansion House)

Session 1 (S1). Lincoln Room, Mansion House
SENsys. Chair: Sid Thomas (Aberystwyth, UK)

09.00 – 09.40 **Susheng Gan (Cornell University, USA)**
Translational research on plant senescence: from bench to bank

09.40 – 10.20 **Vicky Buchanan-Wollaston (University of Warwick, UK)**
Network interference to identify regulators of Arabidopsis leaf senescence

10.20 – 10.50 Coffee/Tea break

10.50 – 11.30 **Paul Dijkwel (Massey University, New Zealand)**
Evolution of ageing in plants

11.30 – 12.00 **Haya Friedman (The Volcani Center, Israel)**
Mitochondrial ROS-related events occurs early during extended darkness as determined by analysis of microarray and reduction-oxidation sensitive GFP

12.00 – 12.20 **Discussion topic**
Phenotypic analysis of senescence – generating quantitative data for mod elling

12.20 – 13.20 Lunch in Old Lounge, Mansion House

Session 2 (S2). Lincoln Room, Mansion House.
EcoSEN. Chair: Tony Stead (Royal Holloway University of London, UK)

13.20 – 14.00 **Marco Archetti (Harvard University, USA)**
On the adaptive value of autumn colours: photoprotection or coevolution? Evidence from the domestication of apple.

14.00 – 14.40 **Helen Ougham and Luis Mur (Aberystwyth University, UK)**
The real reason why leaves turn red in autumn?

14.40 – 15.10 **Lars Björkén (Umeå Universitet, Sweden)**
Girdling and Fertilization Effects on Senescence in European Aspen

15.10 – 15.40 Coffee/Tea break

15.40 – 16.20 **Sergi Munné-Bosch (Universitat de Barcelona, Spain)**
Role of salicylic acid in the regulation of leaf senescence: an ecophysiological perspective.

16.20 – 16.50 **Marta Oňate (Universitat de Barcelona, Spain)**
Centenarians and so young: a relict herbaceous perennial defying ageing

16.50 – 17.30 **Astrid Wingler (University College London, UK)**
Senescence, flowering and temperature networks in annual and perennial herbs

17.30 – 19.00 Posters (P) with beer and wine reception in Old Lounge

19.00 Dinner in Steam Bake and Grill, Executive Centre

Thursday 2nd September

07.00 – 08.30 Breakfast in Steam Bake Grill, Executive Centre

Session 3 (S3). Lincoln Room, Mansion House
ModelSEN. Chair: Paul Dijkwel (Massey University, New Zealand)

09.00 – 09.40 **Klaus Humbeck (Martin-Luther Univ. Halle-Wittenberg, Germany)**
Senescence-specific differential histone modifications and restricting of chromatin in Arabidopsis thaliana and Hordeum vulgare

09.40 – 10.20 **Karin Krupinska (Christian-Albrechts-Universität zu Kiel, Germany)**
Retrograde signalling in the control of leaf senescence

10.20 – 11.10 Coffee/Tea break

11.10 – 11.40 **Jos Schippers (Max Planck, Potsdam-Golm, Germany)**
Cytokinin induced age-signals allow for early ethylene-induced leaf sense cence in Arabidopsis thaliana

11.40 – 12.10 **Basanti Biswal (Sambalpur University, India)**
Reprogramming of sugar metabolism during leaf senescence of Arabidopsis thaliana: Loss in photosynthesis is accompanied by the enhancement in the activity for degradation of cell wall polysaccharides

12.10 – 12.30 **Business meeting**
Location and timing of next SENnet meeting.

12.30 – 13.30 Lunch in Old Lounge

Session 3 (S3) continued. Lincoln Room, Mansion House
ModelSEN. Chair: Karin Krupinska (Christian-Albrechts-Universität zu Kiel, Germany)

13.30 – 14.10 **Amnon Lers (Volcani Centre, Israel)**
Nucleic acid degrading enzymes are involved in PCD associated with se nescence and abscission but do they have additional functions?

14.10 – 14.40 **Salma Balazadeh (Max-Planck-Institut, Potsdam-Golm, Germany)**
Towards gene regulatory networks of senescence controlling NAC factors

14.40 – 15.10 **Anja Smykowski (University of Tübingen, Germany)**
G-box binding factor 1 regulates the onset of leaf senescence in Arabidopsis thaliana and is regulated post-translationally

15.10 – 15.40 Coffee/Tea break

15.40 – 16.20 **Stefan Hörtensteiner (University of Zurich, Switzerland)**
Chlorophyll breakdown: pheophytinase not chlorophyllase cleaves phytol- and forms a multiprotein complex with other chlorophyll catabolic enzymes

16.20 – 17.00 **Hilary Rogers (Cardiff University, UK)**
SAG21: a gene at the interface between senescence and stress responses

17.00 – 19.00 Playtime! Time to make use of the facilities at Wokefield Park. Mini spa packages available, archery, ropes course, giant garden games, golf, tennis, swim, sauna.

19.00 – 19.30 Drinks reception in Maple bar/terrace

(sponsored by the Journal of Experimental Botany)

19.30 Gala Dinner in Lincoln room

Friday 3rd September

07.00 – 08.30 Breakfast in Steam Bake Grill, Executive Centre

Session 4 (S4). Lincoln Room, Mansion House
CropSEN. Chair: Susheng Gan (Cornell University, USA)

09.00 – 09.40 **Cornelius Barry (Michigan State University, USA)**
Senescence and fruit ripening: similar processes but diverse outcomes

09.40 – 10.10 **Carol Wagstaff (University of Reading, UK)**
Manipulating Silique Development in Relation to Whole Plant Resource Allocation

10.10 – 10.40 **Peter Walley (University of Warwick, UK)**
Unraveling the polygenic nature of senescence in Brassica oleracea L. var. talica.

10.40 – 11.10 Coffee/Tea break

11.10 – 11.40 **Laura Graham (R&G Fresh Herbs, UK)**
Pre- and post-harvest challenges that determine the shelf-life and quality of cut fresh herbs.

11.40 – 12.10 **Laia Arrom (Universitat de Barcelona, Spain)**
Interplay between sucrose and hormones in the regulation of floral develop pment in Lilium

12.10 – 12.40 **Tony Stead (Royal Holloway University of London, UK)**
Closing remarks and presentation of prizes kindly sponsored by ***R&G Fresh Herbs, Tozer Seeds and Humber VHB***

12.40 Lunch in Lincoln room

14.00 Depart

Site Information

- An ATM machine is available in the Executive Centre.
- Free Wi-Fi is available in all areas of the conference centre.
- Taxis can be booked through the reception in the Executive Centre.


Key to the Map of Wokefield Park
1 1st Tee
2 Driving Range
3 Executive Centre Reception
4 The Street
5 Swimming Pool & Gym
6 Golf Pro Shop
7 Steam, Bake & Grill
8 Bar (Downstairs)
9 KPMG Hub
10 Nintendo Lounge
11 Archery
12 High & Low Ropes Course
13 The Lake
14 Fishing
15 Football Pitches & Team Building Area
16 Garden Games
17 Crazy Golf
18 The Traditional Entrance
19 Bar
20 Terrace
21 Maple Restaurant
22 Spa
23 The Contemporary Entrance
24 Tennis Court
25 Ivy Cottage
26 BMW Academy & BMW Parking
27 Wellington Lodge
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Mini–Spa Treatment Menu (15-25 Minute Treatments)

Manicure: Shape & Polish only - £14.00 per Person

Pedicure: Shape & Polish Only - £14.00 per Person

Taster Indian Head Massage: £16.00 per Person

Taster Neck & Shoulder Massage: £16.00 per Person

Hand & Arm Massage: £16.00 per Person

Foot & Leg Massage: £16.00 per Person

S1.SENsys

Translational research on plant senescence: from bench to bank

Susheng Gan

Cornell University, Dept of Horticulture, G51 Emerson Hall, Ithaca, NY 14853 USA

The significance of research on plant senescence can not be overemphasized. Leaf senescence limits crop yield and biomass accumulation. About 50% of produce loss is due to senescence. During senescence, various nutrients such as proteins and antioxidants are degraded. Leaf senescence thus causes huge losses economically and nutritionally. Senescence also renders produce susceptible to pathogen attacks, and most postharvest pathogens are fungi that may produce toxic chemicals, rendering food and feed unsafe. Therefore, research and manipulation of leaf senescence will have a profound impact on the national food security in China and worldwide. Research in my lab has been directed to decipher the molecular regulatory mechanisms underlying plant senescence (leaf senescence in particular) in the model plant Arabidopsis and to translate our basic findings into crops for enhanced crop performance in stress environments (such as drought and salt), for increased crop yield and bioenergy production, and for safer food and feed. This talk will summarize our research achievements and progresses in this regard with an emphasis on the unraveling of the senescence-specific AtNAP transcription factor networks from Arabidopsis and how we have translated our basic molecular findings into crops.

S1.SENsys

Network inference to identify regulators of Arabidopsis leaf senescence

Vicky Buchanan-Wollaston, Emily Breeze, Stuart McHattie, Richard Hickman, Andrew Mead, David Wild.

Warwick HRI and Warwick Systems Biology, University of Warwick, Wellesbourne, CV35 9EF

Leaf senescence is a programmed event responding to a wide range of external and internal signals including those caused by development, age and environment. Senescence requires de novo gene expression and protein synthesis and is controlled in a tightly regulated manner. Identification of the genes that control senescence has been complicated by the complex combination of signalling pathways that appear to be involved in senescence. Cross talk exists between senescence and stress or pathogen responses and also the hormonal and nutrient signals that are implicated in the control of senescence.

We are using Arabidopsis as a model, taking a systems biology approach, to identify the genes involved with the control of leaf senescence. Extensive high resolution time course microarray analysis has been analysed using various clustering techniques together with promoter motif analysis to characterise the global changes in gene expression during senescence. We are using a variational Bayesian State Space modelling method and transcriptional networks that pinpoint key regulatory genes operating to control gene expression during developmental leaf senescence have been generated. Mutant analysis with potential hub genes has shown that several of these are important for the normal senescence process and we are building more detailed models around selected hubs. Cross talk between stress related pathways and senescence is being elucidated by the use of mutants, stress treatments and comparative gene expression analysis.

S1.SENsys

Evolution of ageing in plants

Paul Dijkwel and Alvina Lai

Institute of Molecular BioSciences, Massey University, Private Bag 11222, Palmerston North, New Zealand

The harsh reality of life dictates that organisms age. More than a century ago, August Weismann proposed a theory to explain ageing. He partitioned life into two components, the perishable soma and the immortal germ line and somatic vulnerability is the reason why natural selection has made no effort to grant individuals an infinite lifespan. Kirkwood and Holliday's disposable soma theory builds on Weismann's original idea and states that energy is primarily used on reproduction rather than on somatic maintenance. The soma is a transient vessel and the optimal level of somatic repair is lower than what is required to fix all the damage, rendering somatic ageing an unavoidable misfortune.

Age-associated somatic deterioration is obvious in unitary animals but often obscure in single-celled organisms, clonal species and long-lived perennial plants. We propose that all organisms on earth have a short-lived soma vulnerable to deterioration. Our surmise includes the clonal colonies of Quacking Aspen which - some believe - are over one million years old. The key to longevity may be meristematic indeterminacy coupled with asymmetric cell division. Plants seem to have perfected mechanisms of programmed cell death which is most dramatically visualised by autumn senescence. In this manner plants can dispose of accumulated damage and fully rejuvenate each year. We will discuss the proposal that damage control is the primary role of senescence, while an efficient nutrient recovery mechanism evolved as an adaptation.

S1.SENsys

Mitochondrial ROS-related events occurs early during extended darkness as determined by analysis of microarray and reduction-oxidation sensitive GFP

Shilo Rosenwasser[1,2], Ilona Rot[1], Evelyn Sollner[4], Andreas J. Meyer[4], Yoav Smith[3], Robert Fluhr[5], Noam Leviatan[5], and Haya Friedman[1]

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[2]Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Kennedy-Leigh Centre for Horticultural Research, Faculty of Agriculture, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Rehovot, Israel
[3]Bioinformatics Units, Hebrew University, Jerusalem, Israel
[4]Heidelberg Institute for Plant Science (HIP), Heidelberg University, Im Neuenheimer Feld 360, D-69120 Heidelberg, Germany
[5]Plant Sciences, Weizmann Institute of Science, Rehovot, Israel

Bioinformatics and cellular techniques were utilized to establish the occurrence of ROS-related events at early stages of dark-induced senescence. Microarrays of increasing dark durations were analyzed for expression of antioxidants-encoding genes and for the existence of transcriptome signatures common and specific to ROS stresses of subcellular compartments. The redox state was monitored by determining the degree of oxidation of the reduction-oxidation GFP (roGFP) localized to various cellular compartments during darkness[1].

The transcriptome footprint of ROS stress related to cytoplasm and chloroplasts decreased in leaves as early as the second day of darkness. This paralleled an increase in transcripts indicating a general ROS stress, and more specifically an emergence of transcriptome footprint specific to mitochondrial and peroxisome ROS stress. The analysis of roGFP in the different compartments substantiated these results and in plastids the probe degree of oxidation was reduced as early as the first day of darkness followed by a gradual increase. However, in mitochondria the degree of oxidation of roGFP increased as early as the first day of darkness and this was followed by an increase in the peroxisomes and not in the cytoplasm during three days of darkness. Both the roGFP and the microarray analyses showed that a ROS stress emanating from the mitochondria followed by that from the peroxisomes occurs early during darkness and before cell death, suggesting that this can serve as a signal for later deterioration events.

[1] Rosenwasser S et al.(2010) *Physiol. Plant.* 138: 493-502

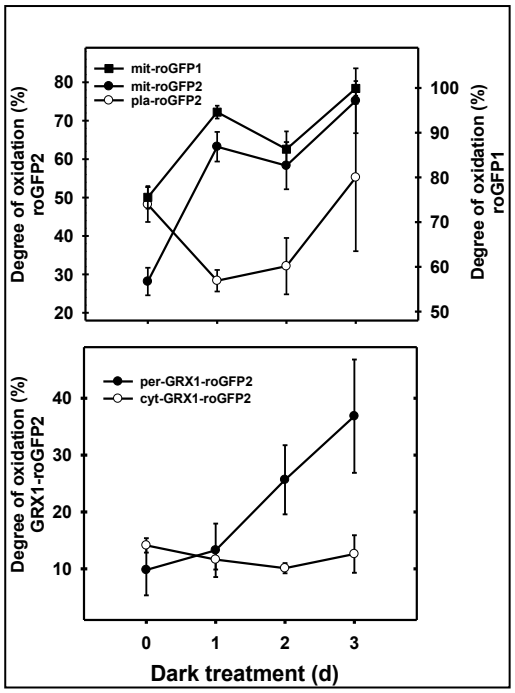

Fig. 1. Changes in the degree of oxidation of roGFP in various cellular compartments during extended dark treatment. Data was obtained from transgenic *Arabidopsis* lines expressing roGFP2 in plastids and mitochondria or roGFP1 in the mitochondria (A), GRX1-roGFP2 expressed in the peroxisome and cytosol (B). Fluorescence related to roGFP was detected at 515 nm following excitation at 400 nm and 485 nm using a fluorometer as described in the Methods.

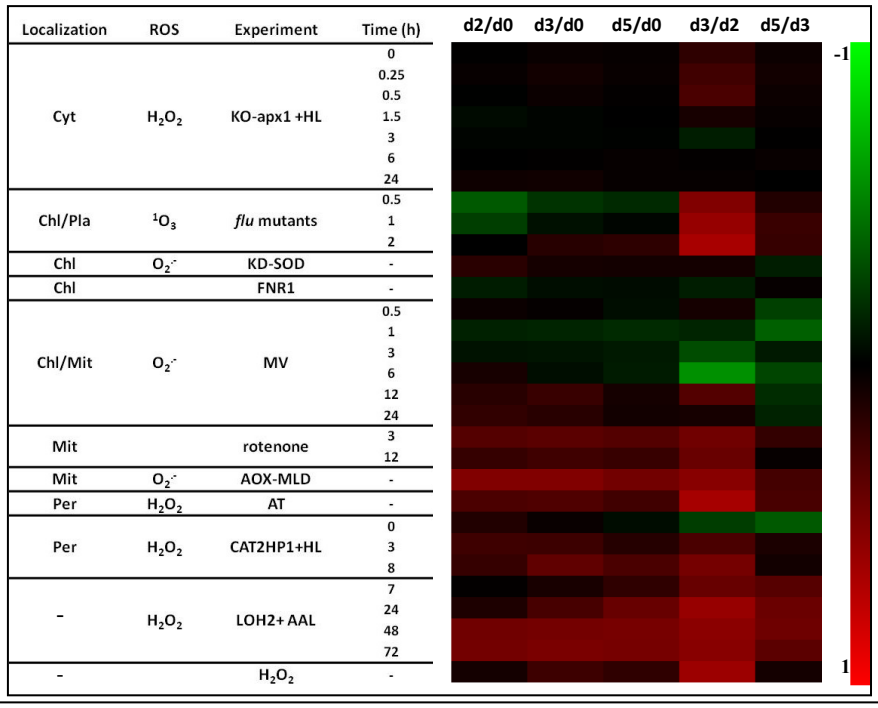

Fig. 2. ROS-related transcriptome signatures under extended darkness. The correlation between gene expression profiles of extended darkness and ROS-related experiments was calculated as described in the Methods and presented as an heat map. The legend to the left describes the localization of ROS, and the possible ROS forms of the 10 different ROS-related experiments and their time points as indicated. The color-coded result for each vector at the various days of dark treatment is shown to the right. Correlation values are between 1 (complete correlation, red) to -1 (highest possible anti-correlation, green). Data for the second day (d2) was normalized to that of non-darkened tissue and data of the third (d3) and the fifth days (d5) were normalized to either that of non-darkened tissue or to that of tissue subjected to darkness for two (d2) or three days (d3), respectively. KO-Apx1-Knockout of Ascorbate Peroxidase 1; HL-high light; flu mutants; KD-SOD-Knockdown of Superoxide Dismutase; FNR1-ferredoxin:NADP reductase; MV-methyl viologen; rotenone-an inhibitor of the Complex 1 in mitochondrial electron transport ; AOX-MLD-alternative oxidase1a mutant plants with moderate light under drought conditions, AT-3-aminotriazole, a potent inhibitor of catalase. CAT2HP1-Catalase deficient plants; LOH2 +AAL-LAG one homolog 2 with the fungal AAL toxin.

On the adaptive value of autumn colours: photoprotection or coevolution? Evidence from the domestication of apple

Marco Archetti

Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

Why do leaves turn red in autumn? While the proximate (biochemical and physiological) explanation of leaf colour change has been known for a long time, we have begun to investigate its adaptive value only in the past ten years. Why are anthocyanins produced in autumn, just before leaf fall? Why only a minority of species turn red in autumn? The two main hypotheses are that anthocyanins function to relieve photo-oxidative stress, allowing a better resorption of nutrients (the photoprotection hypothesis) or that autumn coloration is a warning signal to repel insects that use the trees as a host between autumn and spring (the coevolution hypothesis). I summarize the current state of the debate on the evolution of autumn colours and discuss unresolved issues and future avenues of research[1].

I also present some new evidence on the coevolution hypothesis[2]. First, I show that aphids (*Dysaphis plantaginea*) avoid apple trees (*Malus pumila*) with red leaves in autumn and that their fitness in spring is lower on these trees. Moreover, I show that autumn colours are common in wild populations but not among cultivated apple varieties, which are no longer under natural selection against insects; autumn colours remain only in the varieties that are very susceptible to the effects of a common insect-borne disease, fire blight, and therefore are more in need of avoiding insects. Varieties with red leaves have smaller fruits, which shows that they have been under less effective artificial selection, and suggests a possible trade off between fruit size, leaf colour and resistance to parasites. These observations are consistent with the coevolution hypothesis.

[1] Archetti M. et al.(2009) *Trends Ecol. Evol.* 24: 166-173

[2] Archetti M. (2009) *Proc. Royal Soc. B* 276: 2575-2580

S2.EcoSEN

The real reason why leaves turn red in autumn?

Helen Ougham[1], Howard (Sid) Thomas[1], Amanda Lloyd[1], Carol Wagstaff[2], Stefan Hörtensteiner[3] and Luis Mur[1].

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[2]Food and Nutritional Sciences, University of Reading, Whiteknights, Reading, RG6 6AP, UK
[3]Institute of Plant Biology, University of Zurich, Zollikerstr. 107, CH-8008 Zurich, Switzerland

The striking red, orange and pink coloration developed by many trees and shrubs in temperate regions raises an interesting question: Why should leaves which are entering senescence carry out *de novo* synthesis of anthocyanins, at a time when most other molecules are destined to be dismantled for remobilization of reserves? In recent years the coevolution hypothesis has been extensively discussed in the literature. According to this hypothesis, leaf coloration acts as an "honest signal" to herbivorous insects that the plant is strongly defended, and should be avoided. Other theories include camouflage, fruit flag, antifeedant activity, thermal regulation, and drought resistance (summarized in [1]). However, some of these ideas are unsupported, or actually contradicted, by experimental results.

One explanation which has some support from data is that the anthocyanins have a photoprotective function. As photosynthesis declines during senescence, chlorophyll, with its capacity to generate active oxygen species, becomes potentially dangerous to the leaf. It has therefore been postulated that the anthocyanins are synthesized to act as "sunscreens" for the senescing leaf. But there is a problem with this proposal: the absorption spectra of anthocyanins are very poorly matched to those of chlorophyll a and b, making them apparently unsuited for a photoprotective function. In this paper we present evidence for an alternative role for the red pigments of autumn, obtained using Arabidopsis plants transformed to overexpress anthocyanins in the leaves.

[1] Archetti, M (2008) *Oikos* 118: 328-333

S2.EcoSEN

Girdling and fertilization effects on senescence in European aspen

Lars Björkén, Erik Olofsson and Stefan Jansson

Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, SE–90187 Umeå, Sweden

The process of autumn senescence in deciduous trees is a survival strategy deployed to recover nitrogen and other nutrients from the leaves and store it in the bark and roots so that it can be used again for the next growth period. A considerable amount of the nutrients in the tree is located in the leaves during the growing season, and all which is still in the leaves at abscission is lost to the tree and must be replaced from the soil to maintain the same amount of growth.

We have established that a particular aspen tree (*Populus tremula*) on Umeå University campus initiates autumn senescence around the same date every year, provided that the tree has developed “competence to senesce”, a competence that seem to coincide with growth arrest.

Here we show that aspens can shift the onset of senescence depending on nitrogen availability. In a low nitrogen environment senescence starts earlier in the season, proceeds relatively slow and has strong anthocyanin expression, leading to more complete nitrogen recycling. In contrast, a nitrogen rich environment causes a delayed onset of senescence with less complete nitrogen recycling and no rise in anthocyanin levels.

We also show that girdling of mature trees results in an earlier onset of senescence and an increase in the expression of anthocyanins. The response is similar to the effects of nitrogen starvation, and the girdled trees recover more nitrogen from the leaves than untreated controls.

Our interpretation of this data is that the carbon and nitrogen status, or perhaps their interaction, modifies the trees “competence to senesce” and makes it unable to respond to the photoperiodic cues typically initiating autumn senescence.

S2.EcoSEN

Role of salicylic acid in the regulation of leaf senescence: an ecophysiological perspective

Sergi Munné-Bosch

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Leaf senescence is a highly regulated physiological process that leads to leaf death and is, as such, the last developmental stage of the leaf. Plant reproduction and environmental stresses may induce the process of senescence[1]. Here I will focus on the role of leaf senescence in field-grown plants as a response to adverse climatic conditions and, more specifically, on how it contributes to plant survival under drought stress[2]. Also, I will discuss recent research in which salicylic acid-deficient *NahG* transgenic lines and *sid2* mutants have been used to evaluate the role of this compound in the development of the short-lived, annual plant *Arabidopsis thaliana*, with a particular focus on the interplay between salicylic acid and other phytohormones. It has been found that low salicylic acid levels result in increased growth and delay the progression of leaf senescence leading to smaller abscisic acid levels and reduced damage to PSII (as indicated by F_v/F_m ratios) during the reproductive stage in rosette leaves of *NahG* transgenic lines and *sid2* mutants, compared with wild-type plants. Furthermore, salicylic acid deficiency improves seed yield and composition[3]. The biological significance of these results will be discussed from an ecophysiological perspective.

[1] Munné-Bosch S (2008) Trends Plant Sci. 13: 216-220
[2] Abreu ME, Munné-Bosch S (2008) Env. Exp. Bot. 64: 105-112
[3] Abreu ME, Munné-Bosch S (2009) J. Exp. Bot. 60: 1261-1271

S2/P03.EcoSEN

Centenarians and so young: a relict herbaceous perennial defying ageing

Marta Oñate[1], M. Begoña García[2] and Sergi Munné-Bosch[1]

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[2]Instituto Pirenaico de Ecología (CSIC), Apdo. 13034, 50080 Zaragoza, Spain

Little is known about ageing in perennial herbaceous plants and studies on the hormonal regulation of this process are practically nonexistent. This study was conducted in *Borderea pyrenaica* Miégeville (Dioscoreaceae), which is a small dioecious geophyte endemic to the central Pyrenees (north-eastern Iberian Peninsula). This species is relict from Tertiary and it is one of the longest life spans recorded for any herbaceous plant (more than 350 years). At the end of each growing season, a scar is left on the tuber, which allows the exact determination of the age of individuals. The present study was aimed at examining two of the most important theories of aging in humans, the hormonal and the free radical theories of ageing. Biomass allocation and reproductive effort were measured in parallel with endogenous levels of cytokinins (zeatin, zeatin riboside and isopentenyladenosine), indole-3-acetic acid and abscisic acid which were estimated by UPLC-MS/MS and oxidative stress parameters, both in leaves and etiolated stems. Results demonstrate that centenarians show no signs of senescence and maintain intact their capacity of vegetative growth and reproductive potential. No hints of oxidative stress were observed at old ages, while there were sex-related differences in the hormonal balance of leaves. Endogenous concentrations of cytokinins in leaves decreased with age, but only in females. Such sex-related differences, however, were not associated with leaf senescence, but with an increased reproductive effort in females, although both sexes maintained their reproductive biomass production unaltered at old age. It is concluded that *B. pyrenaica* does not show signs of senescence at the organism level. Females show altered cytokinin levels during maturity, which is associated with their reproductive effort, rather than to a senescing (degenerative) process. The alternate use of five stem cells (meristems) along their life could explain, at least partly, the extraordinary longevity of this species.

S2.EcoSEN

Senescence, flowering and temperature networks in annual and perennial herbs

Astrid Wingler[1], Fabien Chardon[2] and Céline Masclaux-Daubresse[2]

[1] Genetics, Evolution and Environment, University College London, Gower Street, London. WC1E 6BT, UK

[2]Unité de la Nutrition Azotée des Plantes, UR511, INRA Versailles, Route de St Cyr, F-78000 Versailles, France

QTL analysis suggests that flowering-dependent and –independent pathways are involved in the regulation of senescence Arabidopsis[1]. A survey of Arabidopsis mutants in the timing of senescence and/or flowering supports this view, but the majority of mutants for which flowering and senescence phenotypes have been described show either delay or acceleration of both traits. To identify pathways involved in senescence and flowering regulation, transcriptional networks were constructed using transcription factors playing a role in the regulation of both traits as baits. Gibberellin signalling was identified as a possible link between the flowering and senescence pathways. Flowering and senescence in Arabidopsis are under the control of growth temperature, through the effect of vernalisation on flowering[1] and through a senescence- and flowering-delaying effect of cold acclimation[2,3]. Construction of a network underlying in the delay of senescence by cold acclimation suggests a role for light-dependent flowering regulation in this process.

In contrast to the annual plant, Arabidopsis, the perennial plant, *Arabis alpina*, does not die after flowering, but can produce vegetative branches for continued growth. In this species, which is adapted to cold winter temperatures, sudden cold stress accelerates senescence, but continued growth at cold temperature does not. Senescence regulation will be compared (i) in flowering and vegetative branches to determine the interaction of flowering and senescence and (ii) in plants from different altitudes to analyse the importance of adaptation to growth temperature for senescence regulation.

[1] Wingler *et al.* (2010) New Phytol. 185: 420-433)
[2] Masclaux-Daubresse *et al.* (2007) *Plant Physiol.* 143: 434-446
[3] Sharabi-Schwager *et al.* (2010) *J. Exp. Bot.* 61: 261-273

S3.ModelSEN

Senescence-specific differential histone modifications and restructuring of chromatin in *Arabidopsis thaliana* and *Hordeum vulgare*

Klaus Humbeck, Nicole Ay, Ria Uhlemann, Bianka Janack, Gunter Reuter, Andreas Fischer and Kristina Irmler

Institute of Biology, Martin-Luther-University Halle-Wittenberg, Weinbergweg 10, D-06120 Halle, Germany

Leaf senescence involves massive reprogramming of gene expression. Recently it could be shown that in addition to regulation by *trans* acting factors, also epigenetic mechanisms control this process. Our work aims at elucidation of the underlying mechanisms in both, the model plant *Arabidopsis thaliana* and the economically highly important crop *Hordeum vulgare*. Global changes in chromatin structure at the onset of leaf senescence are detected by immunocytology using antibodies against specific histone modifications which determine either transcriptionally active euchromatic or transcriptionally inactive heterochromatic stages. In addition, senescence-specific changes in local epigenetic indexing at central senescence-associated genes (SAGs) was analyzed via ChIP and quantified by real-time PCR. These data clearly show senescence-specific histone modifications at these SAGs. To identify epigenetic factors playing a role in epigenetic control of leaf senescence we screened a set of mutant lines for changes in the course of leaf senescence. Three of them revealed a senescence phenotype. While overexpression of a histone methyltransferase and knock out of a histone deacetylase delay leaf senescence, knock out of the chromatin remodelling factor ddm1 results in an accelerated leaf senescence. In all mutants expression of SAGs was affected, but not that of senescence down regulated genes (SDGs). This indicates that expression of SAGs is under epigenetic control, but not down regulation of SDGs.

S3.ModelSEN

Retrograde signalling in control of leaf senescence

Karin Krupinska

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Plant development requires the coordinated expression of the nuclear and two organellar genomes. Anterograde signalling from the nucleus involves many proteins controlling the different processes of organellar gene expression. Retrograde signalling from plastids and mitochondria, respectively, involves metabolites and reactive oxygen species. During senescence the decline in the functionality of the photosynthetic apparatus precedes the changes in nuclear gene expression. This suggests that nuclear gene expression is affected by signals originating from chloroplasts. Accordingly, adverse environmental situations sensed by disturbances in photosynthetic electron transport and enhanced production of ROS induce senescence prematurely.

It is hypothesized that a high functional status of the photosynthetic apparatus represses senescence associated gene expression in the nucleus. In barley Whirly1 was identified as a factor binding to the promoter of the senescence associated gene *HvS40* which is a typical senescence associated gene induced by SA, JA, ROS and fungal pathogens[1]. Intriguingly, Whirly1 is located in chloroplasts and the nucleus of the same cell[2]. Senescence is altered in transgenic barley plants knocked down in expression of either the *Whirly1* gene or the *HvS40* gene. A model is presented on Whirly1 and HvS40 proteins as a coupled system transducing developmental and environmental information from the chloroplast to the nucleus and regulating senescence associated gene expression.

[1] Krupinska et al. (2002) *Plant Physiol* 130: 1172-1180
[2] Grabowski et al. (2008) *Plant Physiol* 147:1-5

S3.ModelSEN

Cytokinin induced age-signals allow for early ethylene-induced leaf senescence in *Arabidopsis thaliana*.

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Ageing is the universal fate of all living organisms which ultimately leads to death[1]. The observation that single gene mutations can substantially alter life-span reveals that ageing is under genetic control[2]. Senescence is the last stage of leaf development and is a valuable process in the reutilization of nutrients from the senescing leaves to other parts of the plant. Extensive studies on *Arabidopsis* revealed that leaf senescence is a well-defined developmental program. In our research we aim at identifying the age-related changes (ARCs) that precede visual leaf senescence[3]. A mutant screen identified the early ageing mutant *old9* (*onset of leaf death 9*). Transcriptome analysis of *old9* revealed that 90 genes are differentially expressed during standard growth conditions when compared to the wild type. 70% of the differential expressed genes are cytokinin responsive, suggesting that the early ageing phenotype of *old9* may be caused by altered cytokinin homeostasis. This was a surprising finding since cytokinin is well known for its ability to delay the onset of senescence and prolong life-span of leaves. Interestingly, application of exogenous cytokinin to wild type plants during early seedling development results in a partial phenocopy of the *old9* mutant. Map based-cloning of *old9* identified a point mutation in a gene encoding for an apoplastic N-terminal peptide carboxylase-like gene (DUF239). The *old9* mutation is dominant, suggesting that the mutation caused a gain of function. The old9 gene is cytokinin responsive and differentially regulated during the cell cycle[4].Taken together, the *old9* mutant provides a link between cell cycle and early ageing and is in support of the reproductive - cell cycle theory of ageing.

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Reprogramming of sugar metabolism during leaf senescence of *Arabidopsis thaliana*: Loss in photosynthesis is accompanied by the enhancement in the activity for degradation of cell wall polysaccharides

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Leaf senescence in *Arabidopsis thaliana* is associated with a significant loss in photosynthesis. The loss in photosynthetic production of sugar possibly acts as a signal for the expression of several senescence associated genes (SAGs) associated with the induction of several catabolic processes in search of an alternative source of sugar to sustain the respiration of senescing cells. Expression of SAGs, however, follows a temporal pattern, some are expressed early and some at a late phase of senescence. One of the SAGs (*din2*) is found to be expressed late during senescence. The delayed expression of the SAG, we have shown, to be regulated by calcium signaling[1]. We have further expanded the work to examine the physiological significance of the delayed expression of this particular gene. Interestingly, this SAG (*din2*) codes for β-glucosidase, the enzyme suggested to be weakly bound to the cell wall and is responsible for breakdown of the polysaccharide associated with the wall. The participation of β-glucosidase in the senescence program is supported by the senescence induced increase in its activity[2]. In addition to β-glucosidase, β-glucanase, another hydrolyzing enzyme, bound to the cell wall has also been examined. The activity of β-glucanase like β-glucosidase is also found to be enhanced in the background of loss of photosynthesis during senescence. A significant correlation is established between the loss of photosynthesis and enhancement in the activity of cell wall bound enzyme. Since the cell wall remains intact till the last stage of senescence, it could be the final target of the hydrolytic enzymes, which break down the cell wall polysaccharides to provide respiratory sugars as the possible energy source to execute and complete the senescence program. Our data on the activity of both these enzymes possibly explains the physiological significance of late expression of *din2* gene.

The existing literature on sugar signalling (excess and limiting sugars) involved in regulating senescence is controversial[3]. We rather suggest a reprogramming of sugar metabolism as an adaptation for energy depleting cells when there is loss in photosynthetic production of sugar and the cell wall polysaccharides could be the last source of sugar for senescing cells.

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S3.ModelSEN

Nucleic acid degrading enzymes are involved in PCD associated with senescence and abscission but do they have additional functions?

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The T2 ribonucleases (RNases) and type I, S1-like, nucleases are highly conserved, broadly distributed enzymes, found in a wide variety of organisms. The biological functions of these nucleic acid degrading enzymes is largely unknown and were hypothesized to be involved in senescence and programmed cell death (PCD) processes in plants. We investigate function and regulation of the tomato LX T2/S-like RNase and Arabidopsis BFN1 type I nuclease in senescence and abscission processes. *LX* inhibited plants showed marked delay of leaf/flower abscission which suggested involvement of PCD in this process. This hypothesis is supported by the identification of different cellular, biochemical and molecular characteristics of PCD in the AZ area during the late stages of the abscission. The importance of LX function in PCD processes was further demonstrated. We have found that cell death was induced in tomato leaves by phosphate starvation which was markedly delayed in the *LX*-inhibited plants. Thus, *LX* gene regulation and the consequences of its inhibition indicate that this RNase has an important functional role in senescence and abscission but also other responses of the plant which involves PCD. Analysis of the Arabidopsis *BFN1* promoter regulation revealed specific expression during senescence and PCD-associated processes and identified a DNA segment involved in senescence-specific regulation. To allow cellular localization of BFN1 a chimeric BFN1:GFP protein was constructed which was localized in cytoplasmic thread-like structures which are ER-related. Interestingly these BFN1:GFP containing structures reorganized and accumulated around the nuclei creating a wrapping around it as cells senesced. In cells already showing disintegration of the nuclei the BFN1:GFP protein was found to co-localize with nucleic acids in globules. These observations are in agreement with the suggested function of BFN1 in PCD and senescence processes. Recent results raise the possibility that BFN1 has a function in senescence/ abscission unrelated processes as well.

S3.ModelSEN

Towards gene regulatory networks of senescence controlling NAC factors

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Leaf senescence is a genetically programmed process that occurs during late stages of plant development or can be induced by various abiotic stresses. Transcriptional control mechanisms leading to differential gene expression are believed to play important roles in coordinating the senescence process. Our lab studies the function of transcription factors (TFs) that control gene regulatory network (GRNs) during developmental and abiotic stress triggered senescence in Arabidopsis. We are using a combinatorial approach to identify and unravel the role of transcriptional regulators in this process. Using multi-parallel quantitative RT-PCR we searched for TFs that exhibit differential expression during leaf growth, including the transition from fully mature to early senescent, during stress-triggered senescence and for genes that exhibit altered expression in a functional stay green plant (compared to the wild-type control). Differentially expressed genes serve as ´input´ for further targeted analyses. Our analysis identified more than 30 NAC genes that were gradually up-regulated during natural and abiotic stress (such as salinity)-induced senescence. Moreover we discovered that NAC transcription factors play an important role in transcriptional re-programming of the stay green mutant. We currently concentrate our analysis on the discovery of GRNs of several senescence-associated NACs, using a combination of approaches, including BSSA (to determine TF-preferred binding sites), microarray-based expression profiling of transgenic lines overexpressing individual TFs, and transactivation assays (for *in vivo* confirmation of target genes). Examples will be presented.

S3.ModelSEN

G-box binding factor1 regulates the onset of leaf senescence in *Arabidopsis thaliana* and is regulated post-translationally

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Hydrogen peroxide is discussed as being a signaling molecule in *Arabidopsis thaliana* leaf senescence. Intracellular hydrogen peroxide levels are controlled by the hydrogen peroxide scavenging enzyme catalase in concert with other scavenging and producing systems. Catalases are encoded by a small gene family and the expression of all three Arabidopsis catalase genes is regulated in a senescence-associated manner. *CATALASE2* (*CAT2*) expression is down -regulated during bolting time at the onset of leaf senescence and appears to be involved in the elevation of the hydrogen peroxide level at this time point. To understand the role of *CAT2* in senescence regulation in more detail, we used *CAT2* promoter fragments in a yeast-one-hybrid screen to isolate upstream regulatory factors. Among others, we could identify G-box binding factor 1 (GBF1) as a DNA-binding protein of the *CAT2* promoter. Transient overexpression of *GBF1* together with a *CAT2*:*GUS* construct in tobacco plants and Arabidopsis protoplasts revealed a negative effect of GBF1 on *CAT2* expression. In *gbf1* mutant plants, the *CAT2* decrease in expression and activity at bolting time and the increase in H_2O_2 could no longer be observed. Consequently, the onset of leaf senescence and expression of senescence-associated genes was delayed in *gbf1* plants, clearly indicating a regulatory function of GBF1 in leaf senescence most likely via regulation of the intracellular hydrogen peroxide content.

However, GBF1 itself is not regulated on the transcriptional level during senescence. Thus, post-transcriptional regulation mechanisms have been investigated. Protein-protein-interaction analyses revealed an interaction of GBF1 with itself, GBF2, GBF3 and with the C group bZIP factor 63 both *in vitro* and *in vivo*. DNA binding of GBF1 to the CAT2 promoter is altered upon interaction with other factors and upon phosphorylation by Casein Kinase II (CKII). Furthermore, CKII knock-out plants show altered senescence.

S3.ModelSEN

Chlorophyll breakdown: pheophytinase not chlorophyllase cleaves phytol and forms a multiprotein complex with other chlorophyll catabolic enzymes.

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During leaf senescence nitrogen is recycled from chl-binding proteins and, as a consequence, potentially phototoxic chlorophyll (chl) needs to be detoxified. For this, chl is converted in a multistep degradation pathway to colorless tetrapyrrolic end products, called NCCs. NCCs and all chl breakdown intermediates identified to date, such as pheophorbide, lack the phytol moiety of chl, indicating that dephytylation is an early step in breakdown. For decades, chlorophyllase had been implicated to catalyze phytol hydolysis, but *Arabidopsis thaliana* chlorophyllase mutants degrade chl like wild type, questioning their proposed in vivo role. In a functional genomics approach we identified possible alternative phytyl esterase candidates. Mutations in one of these exhibit a stay-green phenotype after senescence induction. The heterologously expressed protein specifically cleaves pheophytin (Mg-free chl) to pheophorbide but does not accept chl as substrate. We named it pheophytinase (PPH). *pph* mutants accumulate small, but significant amounts of pheophytin, pointing to the in vivo relevance of PPH. Collectively our data imply that pheophytinase not chlorophyllase is active in leaf senescence-related chl breakdown. Consequently, the order of early reactions has to be revised: Mg-removal occurs before phytol cleavage resulting in the following order of intermediates: chl → pheophytin → pheophorbide → → → NCCs. Using bimolecular fluorescence complementation, we further show that several of the chl catabolic enzymes form a multienzyme complex at the thylakoid membrane. This is rationalized by the requirement for metabolic channeling to avoid toxicity of highly photodynamic breakdown intermediates.

S3.ModelSEN

SAG21: a gene at the interface between senescence and stress responses

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SAG21 was identified independently both as a Senescence Associated Gene (SAG) in Arabidopsis and later as a gene related to ROS through yeast complementation experiments. It is expressed transiently, early in leaf senescence, is also up-regulated in leaves by drought, abscisic acid (ABA) and oxidants indicating a role in stress protection. SAG21 encodes a group 3 late embryogenesis abundant (LEA) protein with proposed roles in preservation of protein membrane structure, water-binding, hydration buffering, ion sequestration and in preventing protein aggregation, however its role during leaf senescence is not clear. Phenotypic analysis of SAG21 over-expressors and antisense lines reveal a clear senescence phenotype. Down regulation of the gene induces early senescence whereas up-regulation delays senescence. These effects are independent of flowering time. The predicted protein has a signal peptide locating it to the chloroplast and/or the mitochondria suggesting a role in senescence associated events and reactive oxygen regulation. Using a GFP fusion we have shown that SAG21 is mitochondrially located. To understand the regulation of SAG21 gene expression in more detail, we have transformed *Arabidopsis* with SAG21 promoter- GUS fusions comprising a 1600bp promoter fragment that includes four W-box motifs and a 300bp promoter fragment in which three of the W-box motifs have been deleted. We are currently comparing GUS expression in transgenic lines carrying the two constructs under different stress treatments and during senescence.

S3.CropSEN

Senescence and fruit ripening: similar processes but diverse outcomes

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The ripening of fleshy fruits represents a terminal phase in the life cycle of a plant that helps facilitate seed dispersal and as such is often referred to as a senescence phenomenon. Extensive research over the past thirty years has established that fruit ripening together with leaf and flower senescence are highly regulated processes that are influenced by both developmental and environmental cues. At the biochemical level, both ripening and senescence involve alterations in pigment composition, cell wall metabolism and the production of volatile compounds, although the timing of these processes does not necessarily coincide. Similarly, the onset of fruit ripening and senescence involves common classes of regulatory genes and hormone signals. In particular, the plant hormone ethylene plays a fundamental role in regulating the onset of ripening and senescence in many, but not all, plant species. Progress in understanding the ethylene signaling pathway has largely been achieved through genetic analysis in Arabidopsis. This has led to the development of an ethylene signaling model that appears to be broadly conserved across plant species. However, the emergence of genome sequences for many additional plant species, coupled with functional analysis has revealed subtle yet significant differences exist in how ethylene signals are perceived and transduced in diverse species. These differences could impact upon senescence and ripening processes in diverse species. An overview of the similarity and differences between fruit ripening and senescence, with a focus on ethylene biology, will be presented.

S4.CropSEN

Manipulating Silique Development in Relation to Whole Plant Resource Allocation

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The ability to manipulate where resources are ultimately stored within a plant could positively impact both the yield and nutritional value of seeds. An important component of seeds are the seed storage proteins (SSPs), which represent the main plant protein consumed by man and, in various parts of the world, constitute an essential part of human and livestock diets. SSPs primarily act as a source of amino acids for growth upon germination; hence early plant development and seed nutritional quality are intrinsically linked.

The current study investigated how manipulating the number of *Arabidopsis* reproductive structures per plant via selective stem removal impacted upon pod physiology and protein concentration. Analysis showed that whilst fewer siliques per plant resulted in fewer seeds per pod these seeds were of an increased weight and size; therefore there remains a potential for yield enhancement in Brassicaceous species. Such alterations to seed physiology also correlated with delayed leaf senescence, a likely consequence of having fewer pods acting as sinks for resource allocation. Translation of this knowledge of crop ideotype into Brassica has recently begun, with the use of two mapping populations to define regions of the genome which may regulate senescence and resource allocation. These findings demonstrate the highly plastic nature of *Arabidopsis* resource allocation, a process which if further understood could be used to increase the yield and nutritional quality of commercial Brassica crops.

S4.CropSEN

Unraveling the polygenic nature of senescence in *Brassica oleracea* L. var. *italica*.

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Natural plant senescence is a complex trait that marks the end of a photosynthetically active period in a plants life cycle. This may end with the death of the plant or the beginning of a dormant period. Crop plants that are harvested during their vegetative phase are placed into artificial stress induced senescence resulting from the removal of a water supply. Physiological signs of stress include wilting and rapid yellowing. Broccoli is particularly prone to post harvest yellowing, a major factor that contributes to the short shelf life of this crop. To explore the polygenic nature of this trait, we have trialed lines from a unique 'broccoli x broccoli' doubled haploid (DH) population and mapped significant QTL onto a new *Brassica oleracea* L. var. *italica* linkage map. Two 'days to yellowing' (dty) QTL were identified, these have no significant correlations to QTL for physical traits (head diameter, butt diameter, water loss) therefore the effect was not due to pleiotropy. Using markers linked to the QTL, lines that contained beneficial alleles were selected for our backcross (BC) program. We have generated a further set of DH lines from the BC_1. These lines have been genotyped, and useful recombinants were trialed this year. We are currently at BC_3 in the resource development program, these populations will be used to fine map QTL.

S4/P17 CropSEN

Pre- and post-harvest challenges that determine the shelf-life and quality of cut fresh herbs.

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Fresh cut culinary herbs are increasing in popularity in modern cuisine due to their distinctive flavours, aromas and textures. There is a wide range of herbs available in the UK retail market. However, due to the climatic conditions, the UK relies heavily on imported product, particularly during the winter season, to meet the demand in the market. Given the relatively short shelf-life and delicate nature of fresh cut herbs, improving pre-harvest conditions, the management of temperature and humidity during transportation, as well as processing and packaging will increase the longevity of the products and enhance the consumer experience. In these experiments, we have concentrated on *Ocimum basilicum* and *Coriandrum sativum*, both of which have high economic value in the UK industry. We have investigated the impact of using a recycled substrate, which provides an alternative to peat, on the development, yield, shelf-life, flavour and sensory profiles of UK grown crops. We have also manipulated different aspects of the UK and overseas supply chains through alternative packaging and temperature regimes in order to understand how perturbation affects the quality and longevity of the products.

S4/P18 CropSEN

Interplay between sucrose and hormones in the regulation of floral development in *Lilium*

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Floral senescence is a very important (and unwanted) process for cut flower's commercial market, so much efforts have been focused on research for a better understanding of the keys that modulate the progression of senescence. Although ethylene is one of the most important regulators of floral senescence in several species, *Lilium* flowers are insensitive to ethylene, so their senescence must be regulated by other hormones or by other regulators, such as sugars. In this study we have examined how (i) endogenous levels of sucrose and hormones in different organs (outer and inner tepals, androecium and gynoecium) vary throughout flower development and (ii) exogenous application of sucrose alters flower longevity and endogenous levels of hormones at anthesis. Results show that in all cases, floral organs behave differently from each other in the hormonal orchestration of flower development, anthesis marking a key point in the hormonal regulation of flower development. In addition, sugar addition increases the lifetime of the opened flower until tepal abscission occurs. The effect of exogenous sucrose prolonging senescence cannot be mimicked by exogenous application of zeatin. Endogenous variations of sucrose during natural flower development and the contribution of exogenous sucrose in extending lifetime of opened cut flowers underline the important role of sugars in the regulation of flower senescence.

JUB1, a H_2O_2-regulated NAC transcription factor, negatively controls senescence and constitutes a central element in H_2O_2 signaling

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Leaf senescence is a highly complex, genetically programmed process that constitutes an important step for plant fitness and productivity. In addition to age-related factors, various abiotic stresses are known to affect onset and progression of leaf senescence. We are interested to discover the signaling molecules and regulatory factors involved in the crosstalk between age-dependent and abiotic stress-induced senescence. A candidate signal mediating the regulation of age-dependent/abiotic stress-induced senescence is hydrogen peroxide (H_2O_2). We have recently performed global expression profiling using quantitative RT-PCR and Affymetrix micro-array-based hybridization to discover transcription factors (TFs) undergoing expression changes during natural and salt-induced senescence. Among the responding TF genes, the NAC family was overrepresented. Here, we introduce a NAC TF, dubbed *JUB1,* which functions as a negative regulator of senescence. Stress inducible overexpression of *JUB1* dampens the level of cellular H_2O_2 and increases life span accompanied by an increased resistance to oxidative stress. In contrast, precocious senescence and a lowered tolerance against abiotic stresses were observed in a *jub1-1* knock-down line. Analysis of stress inducible *JUB1* overexpression plants under H_2O_2 and salt stress revealed elevated expression of various reactive oxygen species (ROS)-responsive genes including genes encodinge for heat shock proteins (HSPs). Based on our results we hypothesize that JUB1 constitutes a central regulator of a finely tuned control system modulating the cellular level of H_2O_2, regulating stress adaptation and the entry into senescence.

P02: SENsys

Network Directed Screening to Identify Key Regulatory Genes in Arabidopsis Leaf Senescence

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Leaf senescence is an essential developmental process that involves altered regulation of thousands of genes and changes in many metabolic and signalling pathways resulting in massive physiological and structural changes in the leaf. The regulation of senescence is complex and in spite of extensive work around the world the key regulators of senescence remain to be identified. It is likely that there is not a single factor that controls senescence, rather a complex interrelated network of signals is probably involved.

Recently, attempts to elucidate these complex regulatory networks has led to the evolution of time series expression profiling, whereby global analysis is extended across time resulting in temporal expression profiles for all predicted genes of an organism. Network inference methods have been developed that seek to reconstruct transcriptional regulatory networks using the time series data. Network inference suggests network hubs, often these are transcription factors that exhibit numerous regulatory interactions with other genes within the predicted network. These hubs present good candidates for functional analysis by reverse genetics.

We have used this approach to produce transcriptional networks from our high resolution, highly replicated senescence time series data. We have identified a number of interesting hub genes and are performing high throughput mutant screens to test for altered response in dark induced senescence; a high proportion of these hub gene mutants do show a senescence phenotype. As a result of this network directed screening approach we are now starting to predict putative small-scale networks, further in depth analysis of which should help us to elucidate the key regulatory pathways involved in Arabidopsis developmental leaf senescence.

P04: ModelSEN

The Effect of Ethylene Receptor Mutants on Seed Storage Proteins' Nutritional Quality and Yield

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Ethylene is a gaseous plant hormone known to regulate numerous aspects of plant development and displays a strong association with fruit ripening and the senescence of leaves, flowers and reproductive structures. *Arabidopsis* perceives ethylene through five receptors (EIN4, ETR1, ETR2, ERS1 and ERS2*)* which bind to the hormone via copper cofactors. Ethylene receptor mutants are believed to demonstrate a delayed senescence extending the developmental and photosynthetic period in which to accumulate assimilates such as seed storage proteins (SSPs). Similarly manipulating whole plant resource allocation and the mechanisms co-ordinating resource re-distribution from source (leaves) to sink organs (siliques) could also retard senescence. We hypothesised that similar physiological effects might be observed when either increasing the source:sink ratio or using ethylene receptor mutants to alter the timing of senescence. Both manipulations could positively impact upon the yield and nutritional value of seeds (which in *Arabidopsis* are contained within a silique) as food and feed sources by increasing the concentration of beneficial storage compounds such as SSPs.

The current study investigated and how manipulating the number of reproductive structures (sinks) per plant via selective stem removal impacted upon silique and rosette physiology and compared this to three of the *Arabidopsis* ethylene receptor mutants, *ein4*, *ers2* and *etr2.* Analysis showed that fewer siliques per plant delayed leaf senescence and consequentially resulted in seeds of an increased weight. In contrast the ethylene receptor mutants did not exhibit significant differences in silique physiology compared to the wild type but have distinct phenotypes from each other in terms of seed weight, indicating that the ethylene receptors perform separate roles in hormone perception and the regulation of whole plant resource allocation.

Such findings highlight the importance of extending the developmental period and plasticity that exists in *Arabidopsis* resource allocation, which if simultaneously exploited could increase both the nutritional quality and yield of seeds in commercial crops.

P05: ModelSEN

Physiological function of the urea transporter AtDUR3 during senescence in *Arabidopsis thaliana*

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Next to being the most frequently used form of nitrogen fertilizer, urea is increasingly generated during protein degradation, a process of particular importance for the remobilization and retranslocation of nitrogen during germination or plant senescence. Urea and its downstream catabolite ammonium then serve for the re-synthesis of amino acids and oligopeptides, which are loaded into the phloem. Whether transport and metabolism of urea and ammonium might limit the overall nitrogen retranslocation during nitrogen deficiency-induced or plant age-induced senescence is currently unclear.

As a molecule containing 46% nitrogen, urea might be a suitable candidate for intra-or intercellular nitrogen transport. Urea concentrations in leaf samples of different plant and leaf age showed marked differences after plants turned into generative growth, with low urea concentrations in younger or sink leaves and increasing concentrations in leaves of more advanced leaf or plant age. Simultaneously, mRNA abundance of the high-affinity plasma membrane H^+/urea cotransporter AtDUR3 increased with increasing age of leaf samples. Leaf exudate analysis of mutant lines indicated a contribution of AtDUR3 in maintaining elevated urea concentrations in source leaves as well as an involvement in phloem loading, thereby suggesting a function of AtDUR3 in nitrogen retrieval and retranslocation during senescence.

Further investigations on the role of ammonium and urea transporters in nitrogen retranslocation during leaf senescence in Arabidopsis are currently underway.

Catabolite demethylation during chlorophyll breakdown in Arabidopsis thaliana

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Chlorophyll breakdown during leaf senescence ends in the formation of colorless, nonfluorescent open tetrapyrroles, termed NCCs. NCCs are formed from respective fluorescent precursors, termed FCCs, through acid catalyzed tautomerization after import of FCCs into the vacuoles of senescing cells. Modifications of peripheral side chains as present in NCCs isolated from different plant species are introduced at the level of FCCs in the cytosol. One of these modifications, occurring among others in Arabidopsis thaliana, is the hydrolysis of the carboxymethylester at C13-2 of chlorophyll.

Here we present biochemical and genetic evidence for a single methylesterase, MES16, to catalyze this reaction in Arabidopsis. Recombinant MES16, but not other closely related MES proteins, is able to catalyze methylester hydrolysis from a primary FCC, but not from NCCs. MES16 localizes to the cytosol and MES16 gene expression is up-regulated during senescence. *mes16* mutants accumulate a pattern of FCCs and NCCs which is different from wild type. In particular, large amounts of FCCs accumulate in the mutants, whereas in wild type mainly NCCs can be found. FCC-NCC tautomerization experiments indicate that FCCs containing an intact C13-2 carboxymethylester are tautomerized slower as compared to FCCs with a free carboxyl group. As a consequence, senescent leaves of mes16 are highly fluorescing when observed under UV light.

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Barley Whirly1-RNAi plants show delayed senescence at high light intensity

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Whirly1 has been identified as a factor binding to the promoter of the senescence associated gene *HvS40* in young leaves. Intriguingly, Whirly1 is also located in chloroplasts where it binds to intron-containing mRNA species. By its dual localization in chloroplasts and in the nucleus Whirly1 is an ideal candidate for transduction of information from the chloroplast to the nucleus. Chloroplasts can be regarded as sensors of environmental factors such as light and temperature which are known to have tremendous effects on the functionality of the photosynthetic apparatus. We have prepared transgenic barley plants with a knockdown of the *Whirly1* gene. Three independent transgenic lines showed delayed senescence when they were grown in the glass-house during summer. When the plants were grown during winter in the same glasshouse, no apparent phenotype was observed. To elucidate whether differences in light intensity are responsible for the phenotype, barley seedlings were grown in controlled environmental conditions at 150 mE for 10 days and then transferred to higher light intensities (300 mE, 500 mE). Senescence of primary foliage leaves of Whirly1-RNAi lines was clearly delayed at high light intensity. The results show that regulation of senescence processes need to be analyzed at high light intensity and support earlier findings[1].

[1] Noodén et al. (1996) *Physiol Plant* 96:491-495

P08: ModelSEN

Transcriptome of senescent flag leaves collected in barley fields

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Flag leaves were collected in 2009 from field-grown barley as described previously[1]. To monitor senescence, SPAD measurements were performed. Functionality of the photosynthetic activities were analyzed every second day by gas exchange measurements and dual-PAM analyses. At about 3 weeks after anthesis at June 14 carbon dioxide fixation declined. This decline was preceded by changes in SPAD values, in quantum yields of photosystems I and II, electron transport rate and decline in non-photochemical quenching (NPQ). The decline in PS I quantum yield was accompanied by an increase in PS I donor side limitation whereas the PS I acceptor side limitation remained constant. This suggest changes in PSII to be the first signs of senescence.

Global changes in gene expression were analyzed by hybridization to 44K barley Agilent microarrays with RNA from nonsenescent and senescing flag leaves. Expression of genes known to be associated with barley leaf senescence such as *HvSF6* and *HvS40* were upregulated in senescent leaves. In addition new genes with enhanced expression during senescence were identified, e.g. genes encoding WRKY transcription factors. Senescence associated expression of one of these factors was further confirmed by quantitative real-time PCR. An increase in expression of the WRKY gene preceded expression of the *HvS40* gene. Its mRNA level was increased threefold at June 12 compared to June 8 and thereafter increased dramatically more than 200 fold.

[1] Humbeck K, Quast S, Krupinska K (1996) *Plant Cell Environ* 19: 337-344

P09: ModelSEN

Function of a novel MYB transcription factor for plant growth and senescence

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MYB proteins belong to a large family of transcription factors that play regulatory roles in developmental processes and defence responses in plants. Based on the analysis of the expression pattern using qRT-PCR we have identified a senescence-associated MYB factor – AtMMG-1 (Fig.1). Employing transgenic approaches, it was possible to demonstrate that *AtMMG-1* exerts a major role in regulating plant development. The inhibition of *AtMMG-1* expression by RNA interference or T-DNA insertion results in delayed bolting and increased leaf size (Fig. 2). Overexpression of *AtMMG-1* under the control of estradiol-inducible promoter causes earlier bolting and decreased leaf size (Fig. 3). Additionally, to identify AtMMG-1 target genes, a combination of several approaches is being used (Fig. 4). These include estradiol-inducible *AtMMG-1* overexpression coupled to gene expression profiling using Affymetrix GeneChip-based array analysis, identification of AtMMG-1 consensus binding sites using an *in vitro* assay, and promoter analysis. Genome wide expression analysis revealed that 40% of the genes up-regulated upon *AtMMG-1* induction are known senescence-associated genes (SAGs). These data strongly support the conclusion that *AtMMG-1* plays a role during senescence through regulating a network that includes several known senescence associated genes. Further physiological and molecular studies of *AtMMG-1* transgenics will add information to our understanding of the molecular mechanisms that determine life span in plants.

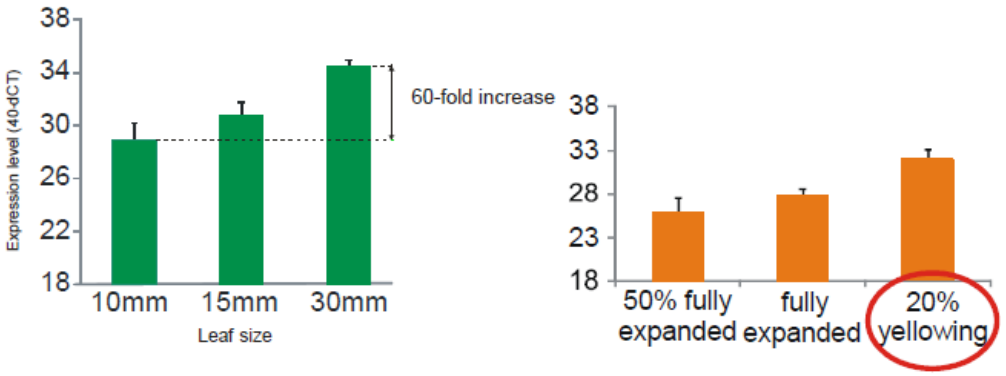

Fig. 1. *AtMMG-1* expression analysis during leaf development (qRT-PCR)

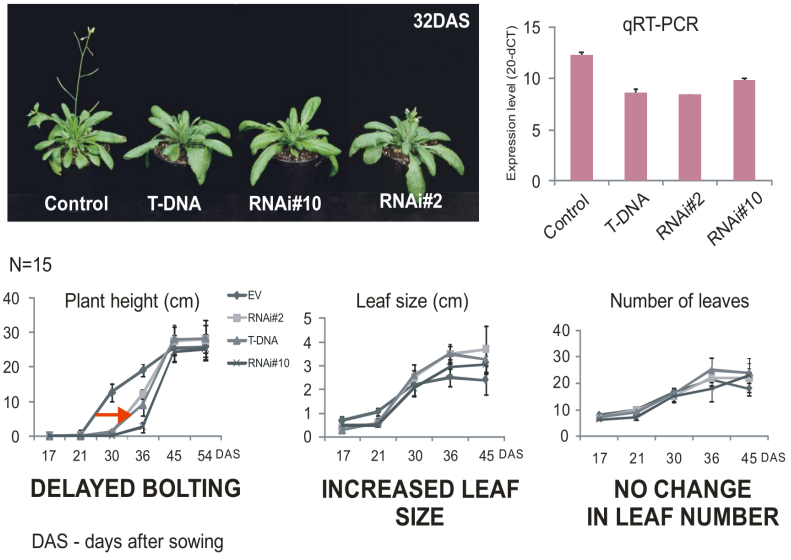

Fig. 2. Analysis of *AtMMG-1* T-DNA insertion lines and RNAi lines

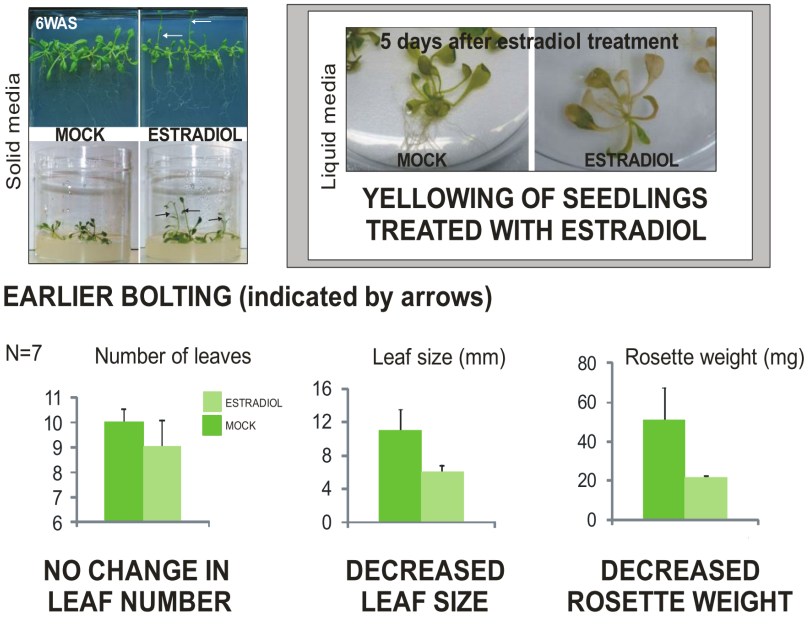

Fig. 3. Analysis of *AtMMG-1* estradiol-inducible plants

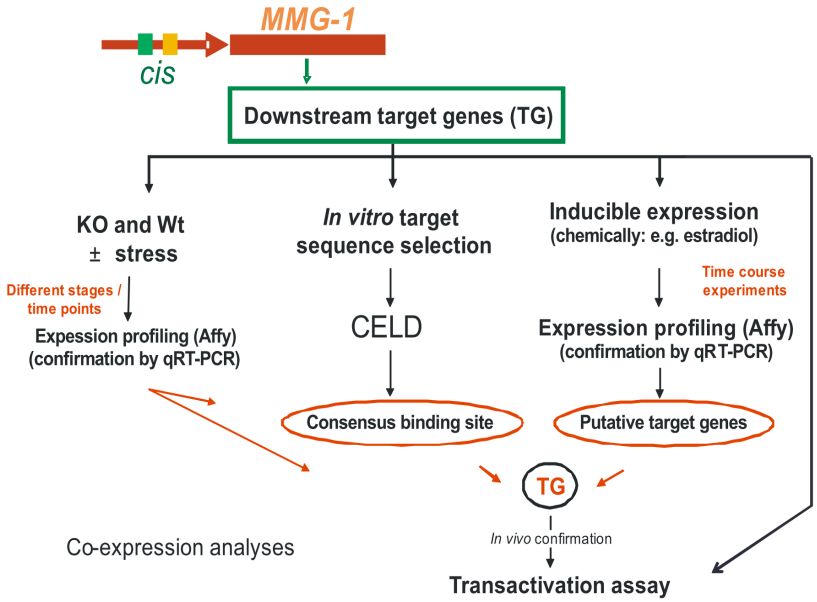

Fig. 3. Identification of AtMMG-1 target genes - workflow

References:

1. Balazadeh S, Riaño-Pachón DM, Müller-Röber, **Transcription factors regulating leaf senescence in *Arabidopsis thaliana*,** Plant Biology 10 (Suppl. 1) (2008) 63–75

P10: ModelSEN

HSN1, a member of the NAC domain family of transcription factors, positively controls H_2O_2-regulated senescence in *Arabidopsis thaliana*

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Leaf senescence is a highly complex, genetically programmed process that defines the final phase of leaf development. Onset and progression of leaf senescence are accompanied by dramatic changes in cellular metabolism. We have recently performed expression profiling using quantitative RT-PCR and Affymetrix micro-array-based hybridization to identify novel transcription factors (TFs) undergoing expression changes during natural and abiotic stress-induced senescence. Many members of the NAC TF family were found to exhibit enhanced expression during senescence, as observed by other researchers before. We started to functionally characterize some selected NAC TFs to study their role for plant senescence. One of the NAC factors, tentatively called HSN1 (for H_2O_2-regulated senescence NAC transcription factor 1) attracted our particular attention. We found that overexpression of HSN1 is lethal to Arabidopsis, while inhibiting its function delayed senescence. To investigate the gene regulatory network controlled by HSN1, we determined its preferred binding site by an *in vitro* approach. To test whether HSN1 functions as a transcriptional activator, a yeast transcription activity assay was performed which indicated that a transcription activation region was located within the C-terminal part of the HSN1 protein. Promoter-reporter (GUS) studies confirmed senescence-dependent expression of the *HSN1* gene. Promoter deletion studies allowed us to identify two highly conserved non-coding sequences. To investigate the role of putative *cis*-regulatory elements involved in oxidative-stress triggered senescence we carried out histochemical GUS assays of *Arabidopsis* plants transformed with *HSN1* promoter deletions of varying lengths, fused to the GUS reporter. We observed an elevated expression of GUS after H_2O_2 treatment. We hypothesize that HSN1 functions as a central regulator of a tightly controlled network of H_2O_2-induced senescence.

P11: ModelSEN

Inferring the signalling network of ANAC092/ORE1: a molecular and functional approach of a leaf senescence regulatory pathway

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Leaf senescence, a developmentally regulated process, contributes to nutrient remobilisation during reproductive growth and finally leads to tissue death**.** This process is trigged by endogenous factors as well as environmental signals and stresses. Transcriptome analysis of leaf senescence in *Arabidopsis thaliana* revealed a complex network of genes involved in the process. ANAC092/ORE1 is a NAC domain transcription factor that shows elevated expression during senescence. It is known that ANAC092/ORE1 plays a pivotal role in controlling leaf senescence but the underlying molecular mechanisms and the pathways it regulates are still only vaguely defined.

Our aim is establish the connection between ANAC092/ORE1 and other regulatory factors that control senescence. To this end, we have initiated a deletion analysis of the *ANAC092/ORE1* promoter; promoter-GUS reporter constructs are currently being tested in transgenic Arabidopsis and tobacco plants. Results will be present. Additionally, we have started to search for direct target genes of the ANAC092/ORE1 transcription factor using genome-wide expression profiling (based on Affymetrix GeneChips) in (a) transgenic plants overexpressing the NAC TF under control of a chemically inducible promoter and (b) Arabidopsis mesophyll cell protoplasts transiently expressing the TF. Candidate target genes will be tested further by e.g. transactivation assays and ChIP (chromatin immunoprecipitation) experiments. To identify proteins interacting with ANAC092/ORE1 we performed a yeast two-hybrid screen. A DNA-binding protein was identified as a potential partner of ANAC092/ORE1.

P12: ModelSEN

MiR840a is involving in the regulation of age-dependent cell death in Arabidopsis leaf

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MiRNAs are a class of non-coding endogenous small RNA, they play important roles in plant development, signal transduction, protein degradation, response to environmental stress and pathogen invasion through posttranscriptional gene regulation by targeting mRNAs for cleavage or repressing translation[1,2]. Here show a miR840a, 21-nucleotides that its target configuration was expressed from the opposite strand of its predicted target gene *AtWhirly3*, which was found within the annotated 3'untranslated region (UTR) of a *ppr* mRNA in the sense orientation[3]. To gain insight into the role of miR840a in the regulation of plant development, two miR840a T-DNA insertion lines were identified as a miR840a knockout line (*miR840a*) and a miR840a overexpression line (*oe-miR840a*) by quantitative RT-PCR. An early yellow senescence phenotype was observed in the *oe-miR840a* line and a staygreen phenotype in the *miR840a* line. And the transcript level of *SAG12* gene was 5.8-fold increased in the *oe-miR840a* line. Although either the presumptive miRNA or its star sequence has a possibility to target to *AtWhirly3* 3' UTR for cleavage, the transcript level of its predicted target gene *AtWhirly3* was 3-fold increased in the *oe-miR840a* line, 2-fold decreased in the *miR840a* line and a *ppr* mRNA level was 198-fold increased in the *oe-miR840a* line and could not detect in *miR840a* line. It indicated that the repressing translation of AtWhirly3 might be connected with a leaf age-dependent cell death while mRNA cleavage of *AtWhirly3* was not regulatory case.

[1] Zhang B et al. (2006) *Dev. Biol* 289:3
[2] Kim JH et al. (2009) *Science* 323:1053
Rajagopalan R et al. (2006) *Genes & Dev* 20:3407

P13: ModelSEN

Chlorophyll breakdown in leaves and fruits: Phytol hydrolysis by pheophytinase

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An early reaction of chlorophyll breakdown during senescence is the hydrolysis of phytol to render chlorophyll catabolites water soluble. Although chlorophyllase (CLH) had been predicted to catalyze dephytylation of chlorophyll in vivo, we recently demonstrated that CLHs are dispensible for leaf senescence in Arabidopsis thaliana. Instead we proposed an alternative route of chlorophyll breakdown, in which dephytylation occurs at the level of Mg-free pheophytin and is catalyzed by a novel senescence-regulated esterase termed pheophytinase (PPH). Here we show that PPH has a high affinity for both pheophytin *a* and pheophytin *b*, but does not accept chlorophylls as substrates. To analyze whether indeed CLH is not active in vivo, transgenic plants were produced which mistarget CLH1 to the chloroplast under the control of the PPH promoter. Expression of Arabidopsis CLH1 in the chloroplast leads to pale-white seedlings indicating that CLHs are not actively metabolizing chlorophyll under natural conditions.

Besides leaf senescence, chlorophyll is also degraded during fruit ripening. Using tomato as a model we aim to investigate the role of PPH in senescence and ripening. In both processes, PPH expression is up-regulated, indicating a role in chlorophyll breakdown in leaves as well as fruits. We started to produce transgenic tomato lines that silence PPH expression constitutively or under the control of a fruit-specific promoter.

Hörtensteiner S (2006) *Annu. Rev. Plant Biol* 57: 55-77
Schelbert S et al. (2009) *Plant Cell* 21: 767-785
Schenk N et al. (2007) *FEBS Lett* 581: 5517-5525

P14: ModelSEN

The nuclear protein HvS40 controls leaf senescence in barley

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The senescence associated gene *HvS40* was shown to encode a 15,4 kDa nucleus targeted protein[1]. Its expression was shown to be enhanced in flag leaves collected from field grown barley[2]. So far, immunological detection of the protein failed indicating a short life time which could be related to its PEST sequence. To get insight into the function of the HvS40 protein transgenic barley plants either overexpressing the gene under control of the maize ubiquitin *UBI-1* promoter or silenced for expression of the gene by RNAi were produced. Plants overexpressing the gene showed a delay of leaf senescence whereas RNAi lines with a knockdown of *HvS40* gene expression showed accelerated senes-cence. These results indicate that HvS40 is a regulator of leaf senescence in barley.

The putative promoter of the *HvS40* gene (840 bp) was used for expression of the *gfp* and the *uidA* gene. We observed that the promoter was only active when the trans-genic barley plants were grown under high light intensity. Moreover we could show that expression of the marker genes did not fully coincide with the expression of the endo-geneous *HvS40* gene. This suggests that the promoter region does not include all elements required for expression of the *HvS40* gene. Nevertheless the promoter might be suitable for senescence regulated expression of genes of interest.

[1] Krupinska K et al. (2002) *Plant Physiol* 130: 1172-1180
[2] Humbeck K, Quast S, Krupinska K (1996) Plant Cell Environ 19: 337-344

P15: ModelSEN

Screening for the mutants with delayed leaf senescence

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Senescence is a very complex process at the cellular, tissue, organ and organismal level leading in the end to death. One of the first symptoms of leaf senescence is chlorophyll degradation appearing as yellowing of the leaves. Senescence is a controlled and coordinated process; however its genetic background is not completely understood. In order to identify genes that are essential for the senescence process we isolated stay-green mutants from EMS-treated seeds. Senescence was induced by darkening of individual leaves using the method as described by Weaver and Amasino (2001) and Keech et al (2007).We have screened about 6000 plants and after using some photosynthesis markers obtained 3 stable, functional stay-green mutant lines (stay-green phenotype was stable after 2-4 cleaning rounds). The phenotype for all of those lines is determined by one gene recessive mutation. Using map base project approach with SSLP and In/Del markers, mutation responsible for stay green phenotype of line 621 have been found on chromosome 3 between markers T20N10 and T8H10, for line 531 on chromosome 1 between markers F24O1 and F4N2. The next step of our experiment will be to connect map based cloning with whole genome sequencing to find the genes responsible for stay green phenotypes.

P16: ModelSEN

Antagonistic senescence regulators Whirly1 and Whirly2 are associated to organelle nucleoids

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Whirly proteins belong to a small family of nucleic-acid-binding proteins. By localization of GFP fusion proteins Whirly1 was shown to be located in chloroplasts and the nucleus while Whirly2 was shown to be imported into mitochondria[1]. Both proteins were shown to bind to RNA in the organelles[2] indicating roles in posttranscriptional processes of organellar gene expression.

Arabidopsis T-DNA insertion mutants of the Whirly genes showed an opposite senescence phenotype. When grown at 70 µE the why1 mutants showed accelerated senescence while in the why2 mutant senescence was retarded. Transgenic Arabidopsis plants overexpressing the Whirly1 gene showed a delayed senescence just as in the why2 mutant. Whirly2 gene expression was shown to be enhanced in the why1 mutant indicating that Whirly1 is a negative regulator of Whirly2.

Transient transformation of mesophyll protoplasts with a 35S-Whirly1-GFP fusion construct revealed that Whirly1 in chloroplasts might be associated to a subpopulation of nucleoids[2]. In mesophyll cells of trans-genic Arabidopsis plants overexpressing a 35S-Whirly2-YFP fusion construct, fluorescence was detected in speckles within slowly moving mitochondria. A model on Whirly dependent coordination of the three genomes within a plant cell during senescence is presented.

[1] Krause et al. *(2005) FEBS Lett* 579: 3707-3712
[2] Melonek et al. (2010) Planta, 232: 471-481

P19: CropSEN

Sustainable production of basil using recycled household compost: impact on flavour and quality

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Basil (*Ocimum basilicum* L.) is a commercially significant crop both in the UK and throughout the World. Since flavour properties of this herb are the primary factor determining its quality, our objective is to improve the sensory characteristics of the product, whilst improving sustainability by reducing energy and chemical inputs. Past research has shown significant phytochemical fluctuations in response to environmental stimuli, such as light, temperature and water. We hypothesised that the growth physiology and phytochemical content of basil is also influenced by nutrient levels and soil quality. We investigated the use of recycled household compost (RHC) as an alternative commercial substrate to soil-based media with the addition of chemical fertiliser. RHC is a widely-available waste by-product from domestic properties which may be beneficial to growers, the consumer and environment. Physiological measurements have been made and have shown that the use of RHC can result in a product with the required aesthetic qualities and yield for commercial use. We have established how the use of RHC substrate during cultivation affects the flavour characteristics of the plant, both *via* GC-MS analysis and human sensory evaluation.

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