Program & Abstracts

9th INTERNATIONAL SYMPOSIUM ON PLANT SENESCENCE

1st - 4th April 2019

Berlin, Germany

Universität Potsdam

Max-Planck-Institut für Molekulare Pflanzenphysiologie

In cooperation with:

agya ARAB-GERMAN YOUNG ACADEMY OF SCIENCES AND HUMANITIES

The conference is hosted by:

University of Potsdam
Max Planck Institute of Molecular Plant Physiology
The Arab-German Young Academy of Sciences and Humanities (AGYA)

In cooperation with:

Acknowledgements:

We acknowledge the generous support of our sponsors and supporters!

Welcome

Dear Colleagues,

On behalf of the Local Organizing Committee and the international Scientific Advisory Committee we are very much honoured to welcome you all at the ´9th INTERNATIONAL SYMPOSIUM ON PLANT SENESCENCE´ here in the heart of Berlin, Germany, right next to the Gendarmenmarkt.

The scientific program assembled by the Scientific Advisory Committee and the Local Organizing Committee includes 13 keynote talks and 33 oral presentations selected from the submitted abstracts. The symposium has been divided into eight sessions covering diverse aspects of plant aging and senescence.

The conference is jointly organized by the University of Potsdam, the Max Planck Institute of Molecular Plant Physiology, and the Arab-German Young Academy of Sciences and Humanities (AGYA).

The University of Potsdam (UP) has been established as a new research and teaching entity shortly after the reunification of East and West Germany. UP is thus a young university, just 27 years of age. The Max Planck Institute of Molecular Plant Physiology is one of the 83 institutes of the Max Planck Society.

The Arab-German Young Academy of Sciences and Humanities (AGYA) is based at the Berlin-Brandenburg Academy of Sciences and Humanities (BBAW) and at the Academy of Scientific Research and Technology (ASRT) in Egypt. It was established in 2013 as the first bilateral young academy worldwide. AGYA promotes research cooperation among outstanding early-career researchers (3–10 years after PhD) from all disciplines who are affiliated with a research institution in Germany or any Arab country. AGYA is funded by the German Federal Ministry of Education and Research (BMBF) and various Arab cooperation partners. Salma Balazadeh, Group Leader of the cooperative research group “Stress Control Networks” at the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany and member of the scientific organising committee of this conference, is AGYA member since 2014.

With a warm welcome,

Salma Balazadeh
MPI of Molecular Plant Physiology, Potsdam, Germany

Bernd Mueller-Roeber
University of Potsdam, Germany

Contents

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Venue

VENUE
The symposium will be held in Berlin, Germany, Berlin-Brandenburg Academy of Sciences and Humanities (BBAW). The BBAW building is located in the center of Berlin and can be easily reached by public traffic (train, flight).

Address
Berlin-Brandenburg Academy of Sciences and Humanities (BBAW)
Markgrafenstrafse 38
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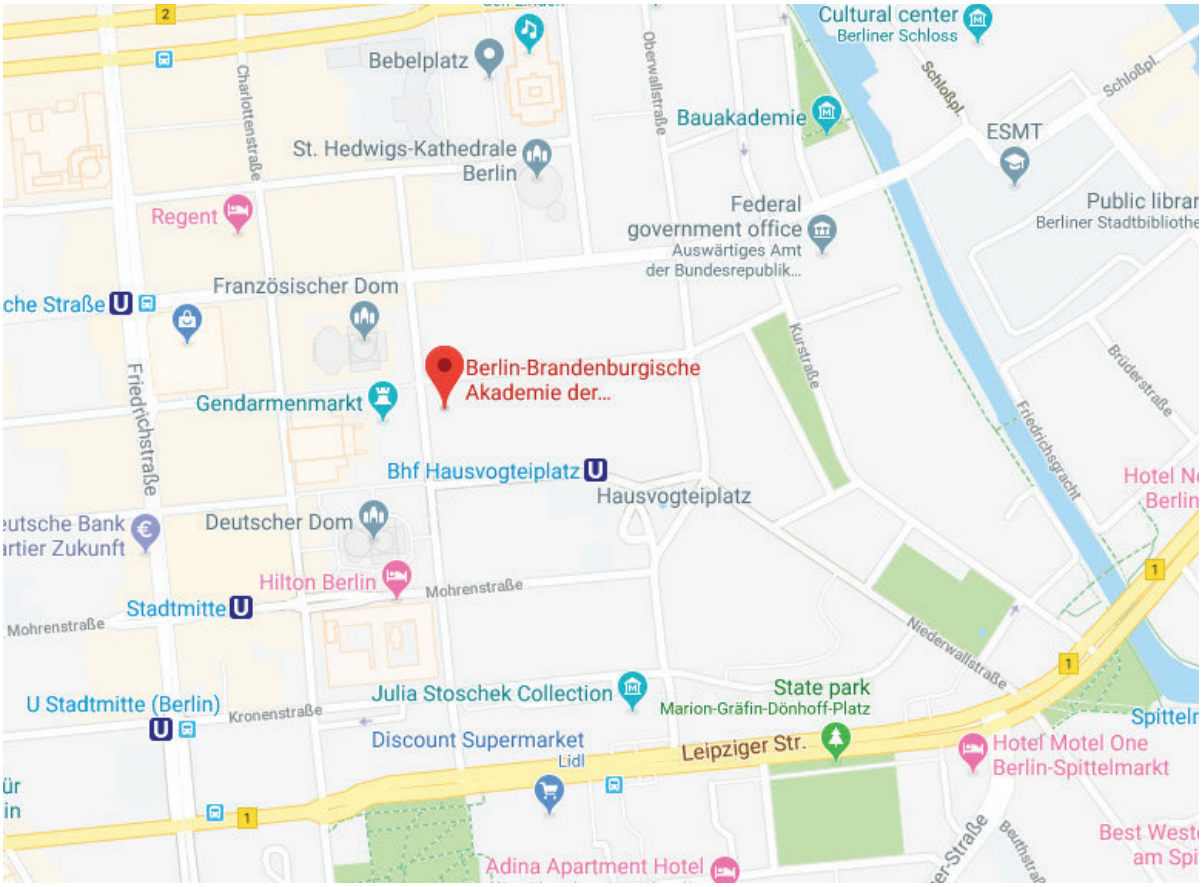

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SCIENTIFIC PROGRAM

April 1, 2019, Monday

10:00-14:00	**Arrival and registration**
14:00-14:20	**Opening remarks**
	SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION
	Chair: **Salma Balazadeh, MPI of Molecular Plant Physiology, Germany**
14:20-15:10	**Hong Gil Nam, Center for Plant Aging Research, IBS, Dageu, South Korea** ***Networks of clocks and senescence and their transition in plant aging***
15:10-15:30	**Sarah Lederer, Freie Universität Berlin, Germany** *Calcium and CDPK in the longevity of plants*
15:30-15:50	**Purva Karia, University of Toronto, Canada** *The mitochondrial tail-anchored protein TTM1 mediates ABA-induced senescence*
15:50-16:20	**Coffee break**
	Chair: **Sucheng Gan, Cornell College of Agriculture and Life Sciences, USA**
16:20-16:50	**Pyung Ok Lim, DGIST, Daegu, South Korea** ***Multidimensional approaches toward understanding leaf senescence: from omics to ecology***
16:50-17:10	**Iman Kamranfar, University of Potsdam, Germany** *NAC transcription factor RD26: a key regulator of catabolism during senescence*
17:10-17:30	**Nico Dissmeyer, Leibniz Institute of Plant Biochemistry (IPB), Halle, Germany** *Conditional protein function via N-degron pathway mediated proteostasis in stress physiology*
17:30-17:50	**Zhonghai Li, Center for Plant Aging Research, IBS, Daegu, Republic of Korea** *ATM suppresses leaf senescence triggered by DNA double-strand break through epigenetic control of senescence-associated genes in Arabidopsis*
18:00-21:00	**Welcome reception at Venue**

April 2, 2019,
Tuesday

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Chair: **Karin Krupinska, Institute of Botany, CAU Kiel, Germany**

9:00-9:50 **Sucheng Gan, Cornell College of Agriculture and Life Sciences, USA**
Making sense and use of senescence

9:50-10:10 **Amnon Lers, ARO, The Volcani Center, Rishon LeZion, Israel**
T2-type Ribonuclease function in ethylene associated processes

10:10-10:30 **Kewei Zhang, Zhejiang Normal University, China**
Feedback induction of salicylic acid hydroxylation serves as a brake in leaf senescence

10:30-11:00 **Coffee break**

Chair: **Pyung Ok Lim, DGIST, Daegu, South Korea**

11:00-11:30 **Hong-Wei Guo, SUSTech, Schenzen, China**
Integrative regulation of plant senescence by an EBFs-EIN3 module

11:30-11:50 **Yongfeng Guo, Tobacco Research Institute, Qingdao, Shandong, China**
The small peptide CLE14 regulates natural and salinity-induced leaf senescence via JUB1-mediated homoeostasis of reactive oxygen species (ROS) in Arabidopsis

11:50-12:10 **Marta Juvany, Umeå Plant Science Center, Umeå, Sweden**
Metabolic adjustments required for extended leaf longevity under prolonged darkness

12:10-12:30 **Rumana Keyani, COMSATS University, Islamabad, Pakistan**
NUCLEOREDOXIN guards against oxidative stress by protecting antioxidant enzymes

12:30-14:00 **Lunch**

SESSION 3: STRESS-INDUCED SENESCENCE

Chair: **Hong Gil Nam, Center for Plant Aging Research, IBS, Dageu, South Korea**

14:00-14:50 **Rashmi Sasidharan, Utrecht University, The Netherlands**
Flooding stress survival: anticipate, acclimate and reanimate

14:50-15:10 **Shimon Gepstein, Kinneret College and the Technion, Israel**

	Releasing the brakes: cytokinins delay senescence and confer extreme drought tolerance by desensitization of environmental clues
15:10-15:30	**Nazeer Fataftah, Umeå University, Sweden** *The capacity of changes in inorganic and organic nitrogen level to influence autumn leaf senescence in aspen*
15:30-16:00	**Coffee break**
	Chair: **Shimon Gepstein, Kinneret College and the Technion, Israel**
16:00-16:20	**Wolfgang Dröge-Laser, Julius-Maximilians-Universität Würzburg, Germany** *The SnrK1-C/S1 bZIP transcription factor network supports metabolic reprogramming and survival during dark-induced senescence*
16:20-16:40	**Vanessa Clouet, Institut de Génétique, Environnement et Protection des Plantes, France** *Involvement of oilseed rape PI-WSCPs "Protease Inhibitors - Water Soluble Chlorophyll Binding Proteins" in nitrogen management and stress tolerance*
16:40-17:00	**Moez Hanin, University of Sfax, Tunisia** *The RSS1-PP1 pathway and its role in plant tolerance to abiotic stresses*
17:00-17:15	**Charlotte Ost, Martin-Luther-Universität Halle-Wittenberg, Germany** *BEP - Barley Epigenome Project*
17:15-17:30	**Christina Mohr, Martin-Luther-Universität Halle-Wittenberg, Germany** *Transcriptomics of stress induced and development dependent senescence in barley*
17:30-19:00	**Poster session**

April 3, 2019, Wednesday

	SESSION 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE
	Chair: **Amnon Lers, ARO, The Volcani Center, Rishon LeZion, Israel**
9:00-9:50	**Mark Aurel Schoettler, MPI of Molecular Plant Physiology, Germany**

	Systems biology of leaf ontogenesis in tobacco – from thylakoid biogenesis to senescence
9:50-10:10	**Shimon Meir, Agricultural Research Organization (ARO), The Volcani Center, Israel** *Cell separation as a final stage of flower senescence: Novelties and challenges in controlling floral abscission*
10:10-10:30	**Nico von Wirén, Leibniz-Institute of Plant Genetics & Crop Plant Research, Gatersleben, Germany** *Characterization of plant age-dependent root senescence in barley*
10:30-11:00	**Coffee break**
	Chair: **Hilary Rogers, Cardiff University, UK**
11:00-11:40	**Remko Offringa, Leiden University, The Netherlands** ***A suppressor of axillary meristem maturation promotes longevity in flowering plants***
11:40-12:10	**Moritz Nowak, VIB, Gent, Belgium** ***KIRA1 and ORESARA1 terminate flower receptivity by promoting senescence-induced programmed cell death in the Arabidopsis stigma***
12:10-12:30	**Annika Wein, Albert-Ludwigs Universität Freiburg, Germany** *Stem cell ageing in the Arabidopsis thaliana root*
12:10-12:30	**Luise H. Brand, Max Planck Institute for Plant Breeding Research, Germany** *Characterization of stem senescence of annual and perennial Brassicaceae species*
12:30-14:00	**Lunch and poster session**

SESSION 5: AUTOPHAGY AND SENESCENCE

Chair: **Nico von Wirén, Leibniz-Institute of Plant Genetics & Crop Plant Research, Germany**

14:00-14:50	**Richard Vierstra, University of Wisconsin, Madison, USA** ***Autophagy, the master of bulk and selective recycling***
14:50-15:20	**Tamar Avin-Wittenberg, Hebrew University of Jerusalem, Israel** ***Autophagy and nutrient remobilization during senescence***
15:20-15:40	**Anne Marmagne, Institut Jean-Pierre Bourgin, INRA, France** *Autophagy and plant proteases for N remobilization during leaf senescence*
15:40-15:55	**Jie Luo, Institut Jean-Pierre Bourgin, INRA, France** *Multiple omics uncover the roles of autophagy on maintaining the*

	balance of endomembrane compositions in Arabidopsis
15:55-16:10	**Venkatesh Thriumalaikumar, MPI of Molecular Plant Physiology, Germany** *Selective autophagy regulates thermomemory in Arabidopsis thaliana*
18:00	**BUS transfer to Potsdam**
19:00-23:00	**Conference dinner, Potsdam, Park Sanssouci**

April 4, 2019, Thursday

SESSION 6: RELATED TALKS

Chair: **Bernd Mueller-Roeber, University of Potsdam, Germany**

9:00-9:50	**Niels Stein, IPK Gatersleben and University of Göttingen, Germany** ***From genome to pan-genome in barley and wheat***
9:50-10:40	**Zach Adam, The Hebrew University, Israel** ***Chlorophyll catabolism precedes changes in chloroplast structure and proteome during leaf senescence***
10:40-11:10	**Coffee break**

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Chair: Moez Hanin, University of Sfax, Tunisia

11:10-11:30	**Karin Krupinska, Institute of Botany, CAU Kiel, Germany** *The senescence associated barley cysteine protease HvPAP14 is targeted to chloroplasts and contributes to the degradation of the photosynthetic apparatus*
11:30-11:50	**Claus-Peter Witte, Leibniz University Hannover, Germany** *Nucleotide catabolism recycles nucleobase nitrogen*
11:50-12:10	**Isabel Schumacher, University of Zurich, Switzerland** *Evolutionary aspects of chlorophyll breakdown*
12:10-12:30	**Su-Hyun Park, Temasek Life Sciences Laboratory, Singapore** *Arabidopsis ubiquitin-specific proteases UBP12 and UBP13 shape ORE1 levels during leaf senescence induced by nitrogen deficiency*
12:30-12:50	**Sichul Lee, Center for Plant Aging Research, IBS, Daegu, Republic of Korea** *Natural variations of the Stay Green gene promoter control lifespan and yield in rice cultivars*

12:50-14:00 **Lunch**

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Chair: **Shimon Meer**, **ARO, The Volcani Center, Rishon LeZion, Israel**

14:00-14:30 **Donald Hunter, The New Zealand Institute for Plant & Food Research Limited, New Zealand**
New insights into the molecular control of postharvest senescence through study of Arabidopsis inflorescences

14:30-14:50 **Lucien Bovet, Philip Morris International (PMI), Neuchâtel, Switzerland**
Metabolic and transcriptomic shifts during tobacco leaf post-harvest senescence

14:50-15:10 **Tie Liu, University of Florida, Gainesville, USA**
Discovering the genes that are involved in postharvest senescence in broccoli (Brassica oleracea)

15:10-15:30 **Sonia Philosoph-Hadas, ARO, The Volcani Center, Rishon LeZion, Israel**
Retardation of cut flower senescence by regulation of anthocyanin pigmentation: Role of light, sugar, and developmental stage

15:30-15:50 **Hilary Rogers, Cardiff University, UK**
Short-term stress affects profiles of volatile organic compounds and gene expression in rocket salad during postharvest senescence

15:50:16:00 **Concluding remarks (Stefan Hörtensteiner)**

ABSTRACTS

Session 1

MOLECULAR LAYERS OF SENESCENCE REGULATION

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

Networks of clocks and senescence and their transition in plant aging

Hong Gil Nam[1,2]

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[2]Department of New Biology, DGIST, Daegu, 42988, Korea

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We previously revealed that ORE1, a NAC transcription factor, forms a trifurcate feed-forward loop that regulates age-dependent cell death. We built time-evolving regulatory networks of NAC transcriptional factors, which show a regulatory inversion from activating to repressive regulatory modes at a pre-senescent transition stage. The inversion was governed by three hub NACs, and the mutants of hub NACs conferred earlier aging. Transcriptomic and proteomic approaches to NACs provide the structure of the regulatory network modules, utilized to negatively control senescence-promoting processes at the leaf transition stage and thus control the timing of age-dependent senescence.

Two rice subspecies, *Indica* and *Japonica*, show drastically different senescence. We found that a quantitative trait locus (QTL) on chromosome 9 is responsible for the short lifespan of *Indica*. The promoter of the *Stay Green* (*SGR*) in the QTL locus induces earlier and higher expression of the gene in *Indica*. The *Japonica SGR* allele introduced into *Indica* varieties led to delayed leaf senescence and increased productivity, suggesting *OsSGR* can be utilized in breeding to improve yield potential.

The circadian clock coordinates physiological processes with daily environmental cycles to enhance the fitness of organisms. To investigate the relationship between clock and aging in plants, we first analyzed potential role of circadian clock in regulating plant leaf aging. Among core clock components, PRR9 makes new-trifurcate feedforward loop for regulating leaf senescence with *ORE1* and *miR164*. Conversely, we found circadian period length is shortened with leaf aging and clock-controlled ORE1 affects this period shortening by interacting with TOC1, a clock regulator.

Leaf senescence is regulated by diverse environmental factors. The red to far-red light ratio (R:FR) is reduced under vegetation shade, thus initiating leaf senescence. We revealed the antagonistic role of phyA and phyB in regulating leaf senescence under FR. Furthermore, we elucidated that the role of phyB in leaf senescence is changed by R:FR. These imply that antagonism between phyA and phyB is involved in fine-tuning leaf senescence under varying FR conditions.

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

Calcium and CDPK in the longevity of plants

Sarah C. Lederer[1], Fabian-Philipp Sylvester[1], Philipp Schulz[2], Anja Liese[1] and Tina Romeis[2]

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[2]Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

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During their lifetime plants undergo diverse physiological changes based on a complex regulatory network. The period of senescence is defined as the last one. Beside of hormones, reactive oxygen species and other regulatory mechanisms, calcium is discussed as a positive regulator in the process of senescence. The underlying molecular mechanism is unknown. Calcium-dependant protein kinases (CDPKs) are known key regulators in signal transduction pathways, which can sense calcium signatures and translate them into specific downstream target phosphorylation.

We identified a CDPK, which functions as a positive regulator of the longevity in Arabidopsis. The *in vitro* biochemical analysis with recombinant, purified enzyme reveals a clear calcium dependency in kinase assay. The K50 for calcium is fairly low indicating it's in vivo function at low changes of the intracellular calcium concentration. Furthermore, single EF hand motifs, the consensus sequence for calcium binding, contribute differently to this low calcium sensitivity.

For this respective enzyme, we isolated CDPK mutant lines and generated overexpression lines. In dark-induced senescence experiments the overexpression lines show a higher chlorophyll content than the wildtype. The mutant lines are characterized by an early senescence phenotype.

We propose that this CDPK may represent a link between developmental calcium signalling and senescence.

Key words: CDPK, Calcium, Longevity, Kinase, Activity measurements

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

The mitochondrial tail-anchored protein TTM1 mediates ABA-induced senescence

Purva Karya[1], Wolfgang Moeder[1], Kazuo Ebine[2], Takashi Ueda[2] and Keiko Yoshioka[1]

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[2] National Institute for Basic Biology. Nishigonaka 38, Myodaiji, Okazaki 444-8585 Aichi, Japan

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Tail-anchored (TA) proteins are a class of proteins that are integrated into the membrane via their C-terminal hydrophobic sequence. These TA proteins perform essential functions on the cytosolic face and are involved in diverse functions including redox reactions, vesicular trafficking and programmed cell death (PCD). The Arabidopsis genome encodes over 150 TA proteins. Arabidopsis TTM1 and TTM2 are TA proteins and belong to the Triphosphate Tunnel Metalloenzyme (TTM) superfamily. Confocal microscopy unveiled sub-cellular localization of TTM1 and TTM2 to the mitochondrial outer membrane. Both, TTM1 and TTM2 possess pyrophosphatase activity in vitro which is unique as a member of TTM superfamily. Our study revealed that TTM2 negatively regulates immunity-related PCD whereas TTM1 positively regulates senescence-related PCD. Promoter swap experiments suggest that the difference in their biological function is governed by their transcriptional regulation. Further, the knockout mutant of TTM1 displays delayed senescence not only during natural development but also upon prolonged dark or ABA treatment. Published phospho-proteomic studies revealed TTM1 phosphorylation at S437 upon ABA treatment. Hence, we have hypothesized that TTM1 phosphorylation upon ABA is important for its function in regulating senescence. We show the importance of TTM1 phosphorylation in regulating senescence. TTM1 phospho-mimic mutants complement the delayed senescence phenotype of ttm1 whereas phospho-null mutants do not complement. Our current data suggests the existence of an ABA-related kinase that phosphorylates TTM1. Together these data indicate that phosphorylation of mitochondrial outer membrane localized TA protein, TTM1, is crucial in regulating ABA-induced leaf senescence.

Key words: Mitochondrial tail-anchored protein, Triphosphate tunnel metalloenzyme (TTM), Programmed cell death, Senescence, Abscisic acid

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

Multidimensional approaches toward understanding leaf senescence: from omics to ecology

Pyung Ok Lim[1], Hyo Jung Kim[2], Jeongsik Kim[2], Hye Ryun Woo[1], Hong Gil Nam[1,2] and collaborators

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Leaf senescence is finely regulated and occurs by an intricate integration of multiple developmental and environmental signals. As a consequence, it is assumed that leaf senescence is a highly complex process involving the collective actions of thousands of genes and multiple pathways associated with aging, as well as their interplays, thereby complicating genetic and molecular analyses of senescence.

To understand leaf senescence, where the influence of many external and internal signals is balanced to allow controlled degeneration of cellular components, it is essential to study the system in its entirety. A highly resolved and multidimensional transcriptome map generated from our RNA-seq data revealed that senescing leaves showed more coordinated temporal changes in transcriptomes than growing leaves, with sophisticated regulatory networks comprising transcription factors and diverse small regulatory RNAs. Furthermore, we also performed comparative transcriptome analyses in genetic mutants, the ethylene-insensitive mutant ein2/ore3 and the constitutive cytokinin response mutant ahk3/ore12, to dissect the role of hormone signaling pathways during leaf senescence. From this study, we found that ethylene acts as a senescence-promoting factor via the transcriptional regulation of stress-related responses, whereas cytokinin acts as an anti-senescing agent by maintaining cellular activities and preserving the translational machinery.

Recently, we have developed a new approach and concept that will facilitate systemic biological understanding of leaf lifespan and senescence, utilizing the phenome high-throughput investigator (PHI) with a single-leaf-basis phenotyping platform. Our pilot tests showed empirical evidence for the feasibility of quantitative measurement of leaf senescence responses and improved performance in order to dissect the progression of senescence triggered by different senescence-inducing factors as well as genetic mutations. Such an establishment enables new perspectives to be proposed, which will be challenged for enhancing our fundamental understanding on the complex process of leaf senescence.

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

NAC transcription factor RD26: a key regulator of catabolism during senescence

Iman Kamranfar[1,2], Mastoureh Sedaghatmehr[2], Alisdair R. Fernie[2], Salma Balazadeh[2] and Bernd Mueller-Roeber[1,2]

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Leaf senescence is a highly regulated process culminating in the degradation of cellular components and massive reprogramming of metabolism to recover nutrients for transport to the sinks. Although transcription factors (TFs) controlling senescence have been identified, TFs regulating the widespread catabolic metabolism occurring during senescence have not been reported. Here, we identify NAC TF RD26 as such a regulator in *Arabidopsis thaliana*. We show that RD26 positively regulates developmental and dark-induced senescence by induction of several senescence-associated genes (SAGs). The RD26 binding site was identified and found to be present in promoters of several RD26-responsive genes most of which play a role in catabolic pathways during senescence. Protein degradation during dark treatment is enhanced in *RD26* overexpression (*RD26Ox*) lines compared to wild type (WT). Our data suggest that RD26 triggers the degradation of chloroplast proteins by direct induction of *CHLOROPLAST VESICULATION* (*CV*) and *SAG15*/*ClpD*. Metabolic profiling indicated different patterns of metabolic alterations during dark-induced senescence in *RD26* transgenic lines compared to WT. In *RD26Ox* lines, accumulation of lysine, GABA, purines and pyrimidines is reduced compared to WT. Our data furthermore indicate an involvement of RD26 in lysine degradation by the direct induction of *LKR*/*SDH*, encoding a bifunctional enzyme catalysing lysine degradation. RD26 also directly regulates *PES1*, encoding the main enzyme in phytol degradation. Degradation of lysine, phytol and GABA has been suggested to be involved in the maintenance of mitochondrial respiration in carbon-limiting conditions. We also report a positive role of RD26 in triggering starch degradation and accumulation of mono- and disaccharides during senescence by the direct induction of *SAG29*, *AMY1* and *SFP1*, involved in carbohydrate metabolism. Collectively, our data suggest that RD26 is a master regulator of primary metabolism reprogramming during senescence.

Key words: Arabidopsis, Dark-induced senescence, Primary metabolism, NAC transcription factor, Mitochondrial respiration

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

Conditional Protein Function via N-Degron Pathway Mediated Proteostasis in Stress Physiology

Reichman Pavel[1], Klecker Maria[1], Naumann Christin[1], Faden Frederik[1] and Nico Dissmeyer[1]

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ETHYLENE INSENSITIVE 2 (EIN2) and RESISTANCE TO DESICCATION 21A (RD21A) are novel putative degradation targets of the N-degron pathway, formerly known as the so-called N-end rule pathway (Dissmeyer, Annu Rev Plant Biol 2019). Transmembrane signal transducer EIN2 is a key element of hormone signaling; cysteine protease RD21A of response to pathogen attack and water deprivation; both also regulate senescence and cell death. The N-degron pathway may relate stability of proteins to the biochemical features of its amino (N)-terminus and its modifications (Dissmeyer et al New Phytol 2018, Dissmeyer et al Trends BiochemSci 2017). Recent discoveries of our and other's lab highlight diverse roles in plant response to environmental stress and in development, namely in cell proliferation and during organ growth (Dong et al. Genes Dev 2017) during plant submergence and under hypoxia (White et al Nature Comms 2017) and possibly in autophagy (Havé et al J Exp Bot 2017). If the N-degron pathway is impaired, plant life is adversely influenced: both biotic and abiotic stress responses like in plant-pathogen/herbivore interaction or under high ambient temperature are negatively affected. We showed that the N-degron pathway is involved in degradation of important regulatory proteins, have developed tools for protein stability surveillance and to study N-degron pathway enzymes (E3 ligases, etc., Mot et al New Phytol 2018), substrate candidates (Naumann et al Meth MolBiol 2016, Reichman et al Meth MolBiol 2017) and protein expression "on demand" as genetic tool for biotechnological applications (Faden et al Nature Comms 2016). We want to understand molecular functions and biological roles of the N-degron pathway by characterizing enzymatic components and physiological substrates and develop biotechnological tools based on targeted proteolysis.

Key words: Ethylene, Cytokinin, RD21A protease, Proteostasis, Ubiquitination, Autophagy

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Oral presentation

ATM suppresses leaf senescence triggered by DNA double-strand break through epigenetic control of senescence-associated genes in Arabidopsis

Zhonghai Li[1,2], JinHee Kim[1], Jeongsik Kim[1,3], Jae Il Lyu[1], Yi Zhang[2], Hongwei Guo[4], Hong Gil Nam[1] and Hye Ryun Woo[1]

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All living organisms are unavoidably exposed to a variety of endogenous and environmental stresses during their lifetimes that can trigger potentially fatal DNA damage, including double-strand breaks (DSBs). Although a growing body of evidence indicates that DNA damage is one of the prime drivers of the aging process in animals, little is known regarding the importance of DNA damage and its repair on lifespan control in plants. Here, we report that the level of DSBs increases but DNA repair efficiency decreases as Arabidopsis leaf ages. Generation of DSBs by inducible expression of an endonuclease, I-PpoI, leads to premature leaf senescence phenotypes. Transcriptome comparison analysis reveals that DSB-inducing conditions cause changes in transcript profiles significantly similar to those that occurred with leaf senescence. Moreover, deficiency in ATAXIA TELANGIECTASIA MUTATED (ATM), the chief transducer of the DSB signal, results in premature senescence in Arabidopsis. ATM interacts with SUVH2 to repress DSB-induced expression of senescence-associated genes, including the genes encoding the WRKY and NAC transcription factors, central components of the leaf senescence process, via modulation of histone lysine methylation. Our work highlights the significance of ATM in the control of leaf senescence, and has significant implications for the conservation of aging mechanisms in animals and plants.

Key words: ATM, DNA double-strand break, Leaf senescence, Histone methylation, SUVH2

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A1

ORESARA15, a PLATZ transcription factor, mediates leaf growth and senescence in Arabidopsis

JinHee Kim[1], Jeongsik Kim[1], Sang Eun Jun[2], Rupak Timilsina[3], Hong Gil Nam[1], Gyung-Tae Kim[2] and Hye Ryun Woo[3]

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Plant leaves undergo a series of developmental changes from leaf primordium initiation through growth and maturation to senescence throughout their life span. Although the mechanisms underlying leaf senescence have been intensively elucidated, our knowledge of the interrelationship between early leaf development and senescence is still fragmentary. Here, we identified the *oresara15-1 Dominant* (*ore15-1D*) mutant which had an extended leaf longevity under natural and stressed conditions and an enlarged leaf size, from activation-tagged lines of Arabidopsis. ORE15 encodes a PLANT A/T-RICH SEQUENCE- AND ZINC-BINDING PROTEIN family transcription factor. A transcriptome analysis in early developing leaves of *ore15-1D* and *ore15-2*, a loss-of-function mutant of *ORE15*, revealed that ORE15 primarily regulates the expression of cell proliferation-mediated growth regulators. ORE15 enhanced leaf growth by promoting the rate and duration of cell proliferation in the earlier stage and suppressed leaf senescence in the later stage by modulating the GROWTH-REGULATING FACTOR (GRF)/GRF-INTERACTING FACTOR regulatory pathway. Our study highlighted a molecular conjunction through ORE15 between growth and senescence, which are two temporally separate developmental processes during leaf life span.

Key words: Leaf senescence, Leaf growth, Cell proliferation, Arabidopsis, PLATZ

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A2

MicroRNA840 positively regulates plant aging in Arabidopsis by interfering with *PPR* and *WHIRLY3* at posttranscriptional and translational levels

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MicroRNAs (miRNAs) play an important role in regulating plant development and in response to environmental cues. It regulates target gene expression by attenuating translation, or by cleaving mRNA, or by working as an enhancer. However, their roles in simultaneously regulating two or more target genes by different action modes is unclear. Here we report a unique miRNA-target configuration, *PPR-miR840-WHIRLY3*, which plays a role in regulating senescence in Arabidopsis. *MicroRNA840* (*miR840*) is located in the overlapping 3'UTR region of the two adjacent genes, *WHIRLY3* (*WHY3*) and a pentatricopeptide repeat (PPR) like protein encoding gene. It can cleave their transcripts within the 3'UTR region in vivo. *MiR840* is highly expressed at the onset of plant senescence and overexpression accelerates senescence process, whereas its suppression displays the inverse phenotype. Using transgenic Nicotiana benthamiana for heterologous expression of *WHY3* or *PPR* coding sequence with and without their 3'UTR or with a mutated 3'UTR in combination with different miR840 expression constructs, we proved that *miR840* represses *PPR* expression by cleaving its mRNA transcripts, whereas WHY3 protein accumulation was attenuated by translation inhibition, as shown by immunodetection and activity assays with a luciferase (LUC) construct c-terminally fused to the WHY3 protein. Interestingly, *miR840* interferes with *WHY3* and *PPR* expression independently and through both targets it synergistically regulates the expression of different subset of senescence-associated genes to regulate plant aging. Therefore, this study demonstrates a new miRNA mechanism to dually regulate its targets via different action modes, and provides a new case study how a miRNA positively functions in regulating senescence in plant.

Key words: Plant senescence, Genetics, Post-transcriptional control, Arabidopsis, MicroRNA

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A3

Transcriptome analysis reveals the involvement of WRKY transcription factors in petal senescence and associated anthocyanin biosynthesis in *Jasminum sambac* flowers

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Being a valued aromatic and ornamental plant, *Jasminum sambac* is widely cultivated in the Southeast Asia for utilization of its flowers. Floral aging in *J. sambac* starts about 24 hours post anthesis (HPA) and ends up in about 36 HPA, with a visual sign of petal-color-turning from white to violet. UPLC-TOF-MS analysis indicated the accumulation of cyanidin 3-rutinoside associated with the aging progression. Floral ethylene release peaked at c.a. 24 HPA correlating with senescence development and color change. To gain more inside into the gene regulation network in this particular flower aging process, we conduct a transcriptome analysis using total RNA samples from petals at 0, 12, 24 and 36 HPA. We identified several transcription factors which were co-expressed well with anthocyanin biosynthesis and ethylene biosynthesis/responsive genes. These included one MYC gene, two bHLH genes and three WRKY genes among others. We isolated the full-length cDNA sequences of these genes and further characterized their functions in transgenic *J. sambac*, petunia and Arabidopsis that together provided primary evidences of a master effect on floral aging by the WRKY transcription factors.

Key words: Floral aging, Transcriptome, WRKY transcription factor, Anthocyanin biosynthesis, Petal

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A4

A tripartite amplification loop involving the transcription factor WRKY75, salicylic acid, and reactive oxygen species accelerates leaf senescence

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Leaf senescence is a highly coordinated, complicated process involving the integration of numerous internal and environmental signals. Salicylic acid (SA) and reactive oxygen species (ROS) are two well-defined inducers of leaf senescence whose contents progressively and interdependently increase during leaf senescence via an unknown mechanism. Here, we characterized the transcription factor WRKY75 as a positive regulator of leaf senescence in *Arabidopsis thaliana*. Knockdown or knockout of *WRKY75* delayed age-dependent leaf senescence, while overexpression of *WRKY75* accelerated this process. *WRKY75* transcription is induced by age, SA, H_2O_2, and multiple plant hormones. Meanwhile, WRKY75 promotes SA production by inducing the transcription of *SA INDUCTION-DEFICIENT2* (*SID2*) and suppresses H_2O_2 scavenging, partly by repressing the transcription of *CATALASE2* (*CAT2*). Genetic analysis revealed that the mutation of *SID2* or an increase in catalase activity rescued the precocious leaf senescence phenotype evoked by *WRKY75* overexpression. Based on these results, we propose a tripartite amplification loop model in which WRKY75, SA, and ROS undergo a gradual but self-sustained rise driven by three interlinking positive feedback loops. This tripartite amplification loop provides a molecular framework connecting upstream signals, such as age and plant hormones, to the downstream regulatory network executed by SA- and H_2O_2-responsive transcription factors during leaf senescence.

Key words: Leaf senescence, Salicylic acid, Reactive oxygen species, WRKY transcription factor, Disease resistance

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A5

Molecular mechanism of histone variant HTB9 regulating leaf senescence and lignin biosynthesis in Arabidopsis

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Leaf senescence is the last stage of leaf development, and macromolecular degradation and nutrient recovery are simultaneously carried out during this process, which affects the yield and quality of crops. Some important regulatory factors were identified by forward genetic screening, indicating that leaf senescence is a genetically regulated programmed cell death process. However, no significant senescence phenotype was observed in most of the loss-of-function mutants of aging-associated genes identified by molecular biology methods, which may be primarily due to functional redundancy. It is very effective to study the function of senescence-related genes by constructing function-acquisition mutants. In this project, by constructing and screening activated tag mutant library, a mutant *HTB9-1D* with premature senescence and increased stalk thickness was obtained, which encodes the histone variant of H2B family and localizes in the nucleus. Transcriptome analysis revealed that the jasmonic acid signaling pathway involved in the regulation of leaf senescence and the phenylpropanoid pathway involved in lignin synthesis were significantly enriched in *HTB9* overexpressing plants, and histochemical staining confirmed the significant increase in lignin content. Taken together, our work provides theoretical basis and technical support for cultivation of bioenergy plant through directional improvement of leaf senescence process and cell wall lignin biosynthesis.

Key words: Leaf senescence, Lignin synthesis, Histone variant, Gene expression regulation, *Arabidopsis thaliana*

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A6

A molecular framework for plant immunity and leaf senescence

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Senescence is the final stage of leaf development and triggered by many intrinsic and extrinsic factors. As a biotic stress, plant pathogens show interactions with host development. Furthermore, an overlap between the pathogen-response and senescence programs is beginning to be characterized. However, the relationship between leaf senescence and plant immunity is unclear. Pipecolic acid (Pip) production via AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1) is further catalyzed by FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1) to produce N-hydroxypipecolic acid (NHP), which is crucial for establishment of systemic acquired resistance (SAR) in Arabidopsis. Here, we characterized that FMO1 is a senescence-associated gene (SAG) and positively regulates leaf senescence in Arabidopsis. Overexpression of FMO1 accelerated leaf senescence in an age-dependent manner, while loss-of-Function of FMO1 delayed this process. Application of Pip could accelerate leaf senescence in wild type and ald1 mutant, but could not act in the fmo1 mutant, suggesting that NHP might play an important role during leaf senescence. Strikingly, the expression of FMO1 was remarkedly regulated by ORE1 and ORS1, which are two key transcription factors involved in regulation of age-dependent leaf senescence, implying a possible role of ORE1/ORS1 in plant immunity. Moreover, we observed that the resistance against Pseudomonas syringae pv. tomato DC3000 in Col-0 increased in senescing leaves compared to mature leaves. Taken together, our work proposes that leaf senescence functions as a positive physiological response for plant immunity, and NHP might be as an important systemic signal molecular to trigger leaf senescence.

Key words: Leaf senescence, Plant immunity, FMO1, ORE1/ORS1, *Arabidopsis thaliana*

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A7

Transcription factor networks regulating *SAG21*: a gene at the interface between stress and senescence

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SAG21/AtLEA5 is a member of the late embryogenesis associated (LEA) protein family. It is expressed strongly in pollen and up-regulated in response to dark, biotic and abiotic stresses in other tissues. It is also expressed transiently during early leaf senescence. Analysis of a 1685 bp upstream region of SAG21 revealed the presence of cis elements including W-boxes, binding sites for WRKY transcription factors, MYC motifs, light regulating elements like GATA box and GT1. Yeast-1-hybrid (Y1H) was used to identify WRKY and NAC family TFs that bind to seven overlapping SAG21 promoter fragments. Thirteen WRKY and four NAC transcription factors bound to the SAG21- promoter. To establish whether the binding was of functional significance, WRKY 15, 33, 63 and 67 were transiently co-expressed with a SAG21 GUS reporter construct in protoplasts. WRKY15 was found to have no significant effect on SAG21 expression whilst WRKY63 induced SAG21 expression by 3-fold compared to GALDB control and WRKY67 caused a significant increase in SAG21 expression. To further demonstrate this regulatory function, real-time qPCR was used to measure SAG21 expression in mutant knockdown lines wrky15, wrky63, wrky67 and nac042 with and without abiotic stresses. Mutation of WRKY15 and NAC042 showed no effect on the SAG21 expression with oxidative stress. However, mutation of WRKY63 and WRKY67 appeared to affect SAG21 expression indicating that they may be direct or indirect regulators of SAG21 expression. To understand further the role of different cis-elements of the SAG21 promoter, a promoter deletion analysis is being performed using overlapping promoter fragments based on the Y1H analysis to drive the GUS reporter gene. The spatial and temporal GUS expression patterns of these constructs are being analysed in response to abiotic stresses and during senescence.

Key words: SAG21, WRKY transcription factors, NAC transcription factors, Arabidopsis, Promoter analysis

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A8

The role of the RNA-directed DNA-methylation (RdDM) pathway in epigenetic control of leaf senescence

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Senescence is the last step in leaf development and is characterized by degradation processes and by the recycling of the leaf resources to other growing parts of the plant, e.g. the developing seeds. During senescence, gene expression is reprogrammed and leads to the induction of SAGs (senescence associated genes) and the repression of SDGs (senescence downregulated genes). Recently, it could be shown that leaf senescence is at least in part under epigenetic control. The epigenetic regulation of gene expression takes place by dynamic alterations in chromatin structure with the participation of different interacting processes like DNA-methylation, histone modification and chromatin remodelling. Depending on the combination of chromatin modifications, the chromatin status can switch between the transcriptionally inactive heterochromatin and the actively transcribed euchromatin. The plant specific RdDM pathway, responsible for the establishing of DNA-methylation in all sequence contexts (CG, CHG, CHH; H=A, T, C) and the maintenance of CHH-methylation, is involved in the formation of heterochromatin and the silencing of transposons. To investigate the impact of the RdDM pathway in regulating leaf senescence, in the model plant *Arabidopsis thaliana* we analyzed on physiological level the course of leaf senescence in different loss of function mutants of key components of the RdDM pathway. In addition, expression of specific SAGs and SDGs was analysed via qRT-PCR.

Key words: *Arabidopsis thaliana* mutants, Epigenetic regulation, Leaf senescence, Reprogramming of gene expression, RdDM pathway

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A9

Link between calcium signaling and sensecence: CPK1 controls senescence master regulator ORE1

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Calcium-triggered intracellular signalling is a prerequisite to mount abiotic and biotic stress responses, but increasing evidence hints toward a role of Ca^{2+}-dependent regulation of plant developmental processes. Calcium-dependent protein kinases combine within one molecule a calcium-sensing domain which contains in general 4 EF-hand calcium-binding motifs and a protein kinase effector domain. Accordingly, CDPKs function as decoders that sense and translate (induced) changes in Ca^{2+} into further downstream signalling events. We conducted conditional in vivo phospho-proteomics screens to identify biological phosphorylation targets of *Arabidopsis thaliana* CDPKs. Here we report on the developmental master regulator of senescence ORESARA1 (ORE1) which was identified by us as a direct in vivo phosphorylation substrate for Calcium-dependent protein kinase CPK1. CPK1-catalyzed phosphorylation of ORE1 triggers transcriptional activation of the downstream target gene BIFUNCTIONAL NUCLEASE1 (BFN1). ORE1 is furthermore required for enhanced CPK1 signaling-induced cell death. Both, CPK1 and ORE1 are mutually required for leaf senescence. Our data implicate that age-related plant cell death is not only controlled by stringent gene regulatory networks through ORE1. But the decision to senesce also contains an additional layer of control towards ORE1 via a post-translational modification that connects to the calcium-regulatory network.

Key words: CDPK, Calcium, Senescence, ORE1, Phosphoproteomics

SESSION 1: MOLECULAR LAYERS OF SENESCENCE REGULATION

Poster presentation: A10

Temporal dynamics of NAC gene regulatory network underlying leaf aging

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'Aging' is defined as gradual changes in an organism over age along their life history, finally leading to age-dependent senescence and death at cell, organ, and organismal levels. Age-dependent senescence involves highly organized regulatory mechanisms as a critical evolutionary strategy for fitness. We have been challenging to uncover the network level mechanisms underlying age-dependent senescence of leaf organ, the critical energy harvest machinery. We previously revealed that ORE1, a NAC transcription factor, forms a trifurcate feed-forward loop that regulates age-dependent cell death. NACs can form dimers and regulate temporal expression of other NACs and senescence-associated genes during leaf aging in Arabidopsis. We built time-evolving genetic regulatory networks of NAC transcriptional factors. These temporal networks revealed a regulatory inversion from activating to repressive regulatory modes at a pre-senescent transition stage from mature to senescent stages. The inversion was governed by three hub NACs, and only the mutants of hub NACs at this stage conferred earlier aging. We further identified potential protein interactors of ANAC090 by using affinity-purification mass spectrometry following isolation of the nuclei of leaf cells at the pre-senescent stage. Collectively, our transcriptomic and proteomic approaches to NACs is providing the structure and function of the regulatory network modules, which is utilized to negatively control senescence-promoting processes at the transition stage of leaf and thus to control the timing of age-dependent senescence.

Key words: Leaf senescence, Time-evolving network, NAC, Pre-senescent repressors, Salicylic acid response

Session 2

HORMONAL AND METABOLIC CONTROL OF SENESCENCE

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

Making sense and use of senescence

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Leaf senescence limits yields in agronomic crops, biomass accumulation in forests, and shelf life of many horticultural crops. A senescing leaf becomes vulnerable to pathogen attack and many pathogens are fungi that may produce toxins, rendering food and feed unsafe. Research in my group has focused on (1) making sense of senescence at the molecular genetics and epigenetic levels. Briefly, we have employed various approaches to the molecular understanding of plant senescence, these include enhancer trap, transcriptome, epigenetics, and bioinformatics. We have identified numerous senescence-associated genes (SAGs) with a focus on genes encoding for transcription factors and genes involved in signal transduction. We have revealed an ABA-AtNAP transcription factor-SAG113 protein phosphatase C-SAG114 kinase regulatory chain, we have also identified recently duplicated transcription factor SAGs that mediate UV-induced senescence. (2) On making use of senescence, we have translated our above basic findings into practical applications for agricultural improvement. We have discovered that using CRIPR/CAS9 system to knock out senescence master regulator in crops can make the crops much more resistant to multiple abiotic stresses such as drought, salt, high temperature, cold, shade and UV. We are especially excited about the UV resistance. For example, lettuce in US and Canada has been repeatedly contaminated by dangerous E. coli strains. One easy way to address the contamination is to use UV to sterilize the lettuce. However, UV readily induces senescence. By using our molecular breeding strategy, we will be able to produce UV-resistant lettuce for consumers. This presentation will review and summarize the two aspects of our researches on plant senescence.

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

T2-type ribonuclease function in ethylene associated processes

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T2-type Ribonucleases (RNases) are RNA-degrading enzymes, which function in various cellular processes. T2-type RNase encoding genes have been identified in various organisms and reach their highest diversity in plants. The existence of T2-type RNase genes in almost every organism suggests they may have an important biological function that has been conserved through evolution. In plants, the T2-type RNases were suggested to be involved in phosphate scavenging and recycling and implicated in defense responses. In non-plant organisms, T2-type RNases were demonstrated to be involved in various important processes and consider to have antitumorigenic & antiangiogenic, as well as pro-apoptotic activities, including ROS propagation during oxidative stress-mediated cell death. Previously, we had shown that suppression of the tomato LX T2-type RNase gene resulted with significant retardation of leaf senescence and flower/leaf abscission. In both of these processes ethylene is well known to have a central regulatory role and cell death processes occur. Further analyses of LX- and LE-suppressed tomato transgenic lines revealed consequences to additional biological processes associated with ethylene regulation including cell death, senescence and response to pathogens. Transcriptomic analysis of tomato LX- or LE-suppressed plants before and after ethylene treatment revealed significant and wide effect resulting with differential gene expression patterns, which significantly differ between wild type transgenic lines. Significant differences in patterns of gene expression between wild type and RNase-suppressed lines are observed already before the ethylene treatment while following the treatment significant inhibition of ethylene-induced gene expression was revealed in the LX-suppressed lines. Preliminary results support an involvement of T2-RNases in the responses to biotic as well as abiotic stresses. The observations regarding the consequences of suppressing T2-RNase gene expression support a central regulatory function for these enzymes. Experiments for investigating LX/LE mode of function, including possible involvement in ethylene response and signal transduction pathways, are in progress.

Key words: T2-RNase, Ethylene, Senescence, Tomato, Programmed Cell Death

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

Feedback induction of salicylic acid hydroxylation serves as a brake in leaf senescence

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The phytohormone salicylic acid (SA) accelerates leaf senescence. Maintaining the SA homeostasis is critical for plants to process the proper leaf senescence. Here we report a feedback induction of SA hydroxylation that maintains the SA homeostasis during leaf senescence of Arabidopsis. We characterized two 2-oxoglutarate Fe(II) oxygenases named as SA 3-hydroxylase (S3H) and 5-hydroxylase (S5H) which convert the SA to 2,3-DHBA and 2,5-DHBA, two major hydroxylated metabolites of SA. The kinetic assays of the two recombinant proteins S3H and S5H show S5H has lower Km than that of S3H, indicating it functions at low-level SA. The qRT-PCR assays show the expression of S5H and S3H were both induced by SA while the S5H was 10-fold more sensitive than that of S3H. The promoter-fused GUS expression patterns show that the S5H expressed from the young stage and was increased during leaf senescence while the S3H specifically expressed during leaf senescence. The individual loss-of-function mutant of s3h and s5h displayed early senescence phenotype. The double mutant of s3hs5h showed dwarf, strong early-senescence phenotype. The metabolic analysis of s3h, s5h and s3hs5h double mutant showed the total SA was over-accumulated at young stage of s5h, senescencing stage of s3h and both young and senescencing stage of s5hs3h, indicating the S3H and S5H corporately maintain the SA homeostasis at different stages. Two members of the SA hydroxylases in Oryza sativa were also investigated and found to play similar roles in maintaining the SA homeostasis. In summary, our research reveals an elegant SA catabolic mechanism by which plants maintain SA homeostasis by converting it to 2,3- or 2,5-DHBA to prevent SA over-accumulation during leaf senescence.

Key words: Metabolic pathway, Salicylic acid hydroxylation, Leaf senescence, Arabidopsis, *Oryza sativa*

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

Integrative regulation of plant senescence by an EBFs-EIN3 module

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Leaf senescence is a highly coordinated, complicated process involving the integration of numerous internal and environmental signals. The plant gas hormone ethylene is a well-known inducer of senescence, including fruit ripening and flower and leaf senescence. As the master transcription factor in ethylene signaling pathway, EIN3 functions as a positive regulator of leaf senescence via regulating multiple downstream target genes, such as miR164 and WRKY75. Recently, we found that an increase in endogenous spermidine through overexpression of spermidine synthase (SPDS) or the exogenous application of spermidine delays leaf senescence by promoting the protein degradation of ETHYLENE INSENSITIVE3 (EIN3) through stabilizing the EIN3 BINDING F-BOX1 (EBF1) and EBF2. Furthermore, the early senescence phenotypes of the spds1spds2 loss-of-function mutant was repressed by ein3eil1 double mutants or overexpression of EBF1/2 that function as negative regulators of leaf senescence. Our results suggest that spermidine could act as an anti-aging signal molecular to antagonize aging pathway in both animals and plants, and plants evolve a unique way to control the aging process via finely regulating the EBFs-EIN3 signaling cascade.

Key words: Leaf senescence; Ethylene; Spermidine; EIN3; EBF

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

The small peptide CLE14 regulates natural and salinity-induced leaf senescence via JUB1-mediated homoeostasis of reactive oxygen species (ROS) in Arabidopsis

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As the last vital step of plant development, senescence of plant organs has been known to be regulated by multiple signals including age, environmental cues, as well as various phytohormones. Here we report that a small secreted peptide, CLAVATA3/ESR-RELATED (CLE) 14, functions in delaying leaf senescence induced by age and salt stress through regulating ROS homeostasis in Arabidopsis. The expression level of CLE14 was induced by developmental age, meanwhile significantly up-regulated by high salinity and multiple stress-related phytohormones including abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA). Synthetic CLE14 mature peptides delayed natural senescence as well salinity and ABA-induced senescence on detached leaves. CLE14 gene knock-down and knock-out plants displayed accelerated progression of natural and salt stress-induced leaf senescence, while gain-of-function of CLE14 delayed these senescence processes. Further analysis showed that CLE14 peptides could regulate ROS homeostasis through inducing transcription of the ROS scavenging genes CAT3, APX1 and APX3. Moreover, CLE14 induced expression of the NAC family transcription factor gene JUB1, which has been previously shown to be involved in senescence regulation and modulation of cellular H2O2 level. Notably, the function of CLE14 peptides in delaying leaf senescence and reducing H2O2 level through activation of ROS scavenging genes was JUB1 dependent. We propose that the CLE14 peptide serves as a novel braking signal in regulating leaf senescence through JUB1-mediated ROS scavenging.

Key words: CLE14, Small peptide, Leaf senescence, Salt stress, ROS, JUB1

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

Metabolic adjustments required for extended leaf longevity under prolonged darkness

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Senescence processes and the efficient nutrient remobilization in the plant are key factors affecting crop yield and post-harvest shelf life. With the aim to better understand the complex regulatory circuits controlling leaf senescence, we performed a forward genetic screen identifying a novel loss-of-function allele of PIF5 (PHYTOCHROME-INTERACTING FACTOR 5) strongly delayed in the induction of senescence in response to prolonged darkness. Even though a crucial function of PIF5 in dark-induced senescence promotion has been demonstrated, and some of the regulatory components have been identified, the metabolic features allowing the mutant leaves to survive in darkness remain unknown. Reduced protein degradation, slight differences in amino acid catabolism together with strong reduced amino acid transport processes and enhanced amino acid accumulation are characteristics defining mutant leaf extended survival in darkness. Our findings suggest that enhanced survival in darkness could be mediated by moderate levels of protein degradation allowing build up and slow usage of amino acids as alternative respiratory substrates. On the other hand, strong degradation processes together with enhanced amino acid transport promote the fast progression of irreversible senescence and antagonize survival. Comparative metabolomics and expression analyses suggest that the senescence regulatory network downstream of PIF5 regulate these irreversible stages through the repression of genes involved in organelle protection and promotion of autophagy together with amino acid export, possibly by the action of senescence promoting factors like ORE1 at their promoters. The failure to induce these later stages may prolong the reversible phase of darkening thus potentially leading to increased viability of individually darkened leaves under darkness for over 2 weeks.

Key words: Metabolism, Dark-induced senescence, PIFs

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Oral presentation

NUCLEOREDOXIN Guards Against Oxidative Stress By Protecting Antioxidant Enzymes

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Cellular accumulation of reactive oxygen species (ROS) is associated with a wide range of developmental and stress responses. Although cells have evolved to use ROS as signaling molecules, their chemically reactive nature also poses a threat. Antioxidant systems are required to detoxify ROS and prevent cellular damage, but little is known about how these systems manage to function in hostile, ROS-rich environments. Here we show that during oxidative stress in plant cells, the pathogen-inducible oxidoreductase Nucleoredoxin 1 (NRX1) targets enzymes of major hydrogen peroxide (H_2O_2)-scavenging pathways, including catalases. Mutant nrx1 plants displayed reduced catalase activity and were hypersensitive to oxidative stress. Remarkably, catalase was maintained in a reduced state by substrate interaction with NRX1, a process necessary for its H_2O_2-scavenging activity. These data suggest that unexpectedly H_2O_2-scavenging enzymes experience oxidative distress in ROS-rich environments and require reductive protection from NRX1 for optimal activity.

Key words: Nucleoredoxin, Thioredoxin, Catalase, Oxidative stress, Reactive oxygen species

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Poster presentation B1

The protein phosphatase PP2A-B′γ negatively regulates developmental leaf senescence by controlling salicylic acid production and signalling in Arabidopsis

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The onset of developmental leaf senescence in Arabidopsis as a consequence of internal and external cues is tightly regulated on the level of the proteome and transcriptome. An important element in this regulation is the transduction of senescence-specific signals by posttranslational modifications, among them phosphorylation. Yet, to date, only few protein phosphatases and kinases have been described which directly influence the onset or progression of leaf senescence, either negatively or positively. We identified the protein phosphatase 2A regulatory subunit PP2A-B'γ as a negative regulator of developmental leaf senescence under short-day conditions. In a knock-down mutant pp2a-b′γ the apical part of older leaves develops premature chlorosis and subsequently cell death spots, which does not occur in a knock-out of the close homologue, pp2ab´ζ. This coincides with strong protein expression of the senescence marker SAG12 and the salicylic acid-signaling marker PR1 as well as the accumulation of salicylic acid in the apical halves of pp2a-b′γ leaves. All of these phenotypes are completely abolished in a pp2a-b′γ sid2 double mutant, indicating that they depend on salicylic acid produced via the isochorismate synthase SID2. RNAseq-analysis revealed that key transcription factors implicated in the initiation of developmental leaf senescence as well as their known target genes and other senescence-associated genes were transcriptionally induced in the knock-down mutant pp2a-b´γ in a SID2-dependent manner. This transcriptional pattern and all other senescence-related phenotypes were abolished in a complementation line expressing 35S:PP2A-B'γ in a genetic pp2ab´γ background. The absence of SAG12, which we identified additionally as an interacting protein of PP2A-B'γ, had no macroscopic impact on the progression of apical leaf chlorosis and cell death in the genetic pp2a-b′γ background. Together, these results indicate that PP2A-B'γ negatively regulates the salicylic acid-dependent onset of developmental leaf senescence in short-day conditions in Arabidopsis.

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Poster presentation B2

SiNAC1 involves in a positive feedback loop via ABA biosynthesis and leaf senescence in foxtail millet

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Leaf senescence, a unique developmental stage involving macromolecule degradation and nutrient remobilization, is finely tuned and tightly controlled by different gene families. NO APICAL MERISTEM, ARABIDOPSIS ATAF1, and CUP-SHAPED COTYLEDON (NAC) transcription factors have been demonstrated to be involved in the modulation of leaf senescence in many land plant species. Foxtail millet (*Setaria italica* L.), an important food and fodder crop, has been studied for its strong stress tolerance and potential to be a biofuel model plant. However, the functional roles of senescence-associated NACs in foxtail millet are still unknown. In this study, we characterized a nuclear localized NAC transcription factor, SiNAC1, which is induced by senescence and concentrated in senescent leaves in foxtail millet. Furthermore, SiNAC1 also positively responds to abscisic acid (ABA) treatment in foxtail millet. Moreover, SiNAC1 promotes the natural and dark-induced leaf senescence by an ABA-dependent manner in *Arabidopsis thaliana*. NCED2 and NCED3 are elevated by SiNAC1 overexpression, which subsequently promotes ABA biosynthesis in Arabidopsis. When the transcription levels were knocked down in foxtail millet by virus induced gene silencing (VIGS) strategy, the senescence process was delayed significantly. Finally, as a homolog of AtNAP, SiNAC1 can partially rescue the delayed leaf senescence phenotype in *atnap* mutants. Overall, our results demonstrate that SiNAC1 functions as a positive regulator of leaf senescence and is involved in a positive feedback loop via ABA biosynthesis and leaf senescence.

Key words: Foxtail millet, Leaf senescence, Transcription factor, ABA

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Poster presentation B3

The HD-Zip class I transcription factor JUB2 modulates growth and senescence by regulating JUNGBRUNNEN1 in Arabidopsis

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The *Arabidopsis thaliana* NAC transcription factor (TF) JUB1 (At2g43000) is a central regulator of senescence and the interplay between growth and stress responses. Employing a yeast-one-hybrid (Y1H) assay and transcript analysis of transgenic lines, we identified an HD-Zip class I TF as a potential upstream regulator of *JUB1*, and we named it JUB2. Here, we characterize the biological functions of JUB2 and unravel its gene regulatory networks. We found that overexpression of *JUB2* results in a GA (gibberellic acid)-deficient phenotype with a compact rosette, shorter stem, impaired filament elongation, fewer siliques and delayed senescence, compared to wild type. These phenotypes are similar to *JUB1* overexpressors. Furthermore, overexpression of *JUB2* in the *jub1* mutant background largely rescued growth and developmental defects, suggesting that JUB2 acts upstream of *JUB1*. We also found that overexpression of *JUB2* or *JUB1* significantly repressed drought induced senescence. By contrast, double mutant lines overexpressing *JUB2* in the *jub1* mutant did not perform better than wild type in response to drought stress, suggesting that the JUB2-*JUB1* cascade is also involved in the drought response. To identify other target genes of JUB2, we performed gene expression profiling by RNA-seq and found *GA2OX* genes to be upregulated by JUB2. GA2OXs are GA 2-oxidases that convert bioactive GAs to inactive isoforms. Indeed, we found that more bio-inactive GAs and less bioactive GAs accumulate in *JUB2OX* plants compared to wild type. Using ChIP-qPCR and gene expression analysis by qPCR, we confirmed that JUB2 directly and positively regulates *GA2OXs*. Taken together, our results suggest that JUB2 controls a complex regulatory system underlying growth and senescence programs.

Key words: GA-deficiency, Transcription factors, Gene regulatory networks, Drought, Senescence

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Poster presentation B4

When and how to senesce? – Exploring metabolic dynamics during autumn senescence in aspen

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European aspen (*Populus tremula* L.) undergoes a highly coordinated senescence program in autumn to remobilize nutrients and to store them in to stem and roots for the winter to be reused the next spring. A timeline for autumn senescence in aspen has been established, but the main triggers for the initiation of senescence are not known. We utilized natural variation in Swedish aspen collection (SwAsp) grown in a common garden to study the timing of autumn senescence and the underlying metabolic readjustments.

The studied six genotypes differed in their autumn phenology displaying early, intermediate or late onset of leaf senescence with a maximum difference of 24 days. As expected, the genotypic variation in the timing of autumn senescence was reflected also in the timing of metabolic changes, i.e. the genotypes with early senescence phenotype showed early accumulation of typical senescence associated metabolite markers, such as aromatic and branched chain amino acids. In addition, high levels of raffinose and orotic acid appeared to be important senescence associated markers in aspen leaves. During senescence, leaf metabolite profile displayed a gradual shift from anabolic to catabolic processes that was represented by the induction of nutrient recycling and transport processes (amino acid and nucleic acid metabolism) and the downregulation of photosynthesis and photorespiration (sugar and glycine metabolism). Dynamic metabolomic analyses also revealed different time-related patterns among the genotypes for example in the levels of pyrimidine metabolites, salicylic acid and sugars. Network analyses were conducted to identify candidate metabolites that could play key roles in the regulation of onset and progression of autumn senescence in aspen.

Key words: Autumn senescence, Populus, Metabolomics, Amino acids, Sugars

SESSION 2: HORMONAL AND METABOLIC CONTROL OF SENESCENCE

Poster presentation B5

Coregulation of the heme scavenger and multistress-induced Translocator protein (TSPO) and the iron-regulated transporter (IRT1) modulates iron homeostasis in *Arabidopsis thaliana*

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The *Arabidopsis thaliana* Translocator protein (AtTSPO), is transiently induced under several abiotic stress conditions including osmotic and salinity stress, and abscisic acid treatment. It was shown that the level of AtTSPO in the cell is tightly regulated, and AtTSPO is actively degraded through a selective autophagy pathway. The endoplasmic reticulum (ER) and mainly Golgi-localized AtTSPO degradation requires heme binding, with the histidine residue at position 91 (H91) acting as heme axial coordinator. We wonder whether heme scavenging by AtTSPO could contribute in iron recycling from the vacuole by the cell and possibly affect iron homeostasis including its uptake. We show in this work that constitutive expression of AtTSPO resulted in Arabidopsis transgenic lines with substantially reduced levels of Iron-Regulated Transporter 1 (IRT1). Interestingly, overexpression of a relatively more stable variant of AtTSPO harboring the point substitution H91A resulted in undetectable levels of IRT1 in the roots of the transgenic seedlings. Bimolecular fluorescence complementation analyses showed that AtTSPO and IRT1 physically interact in the ER and the Golgi membranes *in planta*. Furthermore, we found that AtTSPO is transiently induced by iron deficiency and is constitutively induced in *irt1* null mutant. In addition, overexpression of IRT1 induces AtTSPO expression. An in silico analysis of available expression dataset suggest that in the Arabidopsis root, salinity-induced AtTSPO is correlated to downregulation of genes involved in iron uptake. Likewise, expression of AtTSPO correlates with the upregulation of genes involved in intracellular trafficking of iron. These findings suggest that heme scavenging by the autophagic degradation of AtTSPO contribute to iron recycling, and in particular, the levels of IRT1 in the cell is regulated by that of AtTSPO.

Session 3

STRESS-INDUCED SENESCENCE

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

Flooding stress survival: anticipate, acclimate and reanimate!

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There has been a worldwide increase in intense precipitation events leading to floods. This trend exacerbated by global warming, is expected to persist in the future. Due to the extreme sensitivity of major crops to wet conditions, flooding poses a significant threat to food security. As a result of severely compromised gas exchange in an aqueous environment, flooded plants cannot sustain normal functioning. Flooding is a compound stress involving dynamic changes in oxygen, ethylene, reactive oxygen species and carbohydrates. The spatial and temporal dynamics of these signalling molecules convey important information about the nature of a flooding event and triggers crucial downstream acclimative responses. A comprehensive understanding of the hierarchy and function of these signals, their interaction and the changes they trigger to prolong plant survival in flooded conditions is vital for identifying tolerance strategies. In nature there is a tremendous variation in flooding tolerance both within and across species. This variation can be exploited to identify regulatory genes and networks mediating tolerance and provide possibilities towards improving flood tolerance of sensitive plant varieties. To this end, we have exploited natural variation in wild and model plant species, combining submergence physiology with genome-wide transcriptome and metabolite profiling. In this talk I will illustrate how we have utilised natural variation to unravel the molecular tapestry of flooding responses in plants and the genes and processes that determine the capacity for prolonged survival in flooded conditions.

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

Releasing the brakes: cytokinins delay senescence and confer extreme drought tolerance by desensitization of environmental clues

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In nature, annual plants respond to abiotic stresses by activating a specific genetic program leading to early flowering and accelerated senescence. Overcoming this genetic programing by cytokinins (CK) in transgenic plants that over produce CK (by IPT induction regulated by SARK promoter) has shown a significant increase in plant productivity under drought stress condition. To decipher the regulatory mechanism underlying the phenomenon of cytokinins-induced stress- tolerance, the following analytical approaches and tools were employed: Expression of candidate stress-related genes such as antioxidants and kinases known to be involved in stress tolerance, Analysis of short-term kinase activity in tobacco cell suspension, phosphoproteomics and bioinformatics analysis. The results indicate that components of stress signaling and tolerance pathways known to be activated under stress are slowing down by cytokinins either by adding exogenous cytokinins or by upregulating biosynthesis of endogenous cytokinins. Kinase activity related to abiotic stress was reduced when exogenous cytokinins (BAP) were added to tobacco cell suspension under salt stress conditions. Phosphoproteomics of tobacco cell suspension treated with BAP indicate that more than 50% of the identified phosphoproteins were downregulated under drought stress conditions. We hypothesize that upregulation of cytokinins levels under abiotic stress de-sensitize known signaling pathways that normally are being activated under stress and consequently inhibit growth. Our data suggest that de sensitization of environmental clues prevents growth arrest and allow normal growth and metabolic activities such as photosynthesis under stress conditions.

Key words: Drought tolerance, Cytokinins, Senescence, Environmental stress, Growth inhibition

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

The capacity of changes in inorganic and organic nitrogen level to influence autumn leaf senescence in Aspen

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During autumn, resources like nitrogen (N) are remobilized from senescent leaves and stored in other plants parts such as the bark and roots to be used in the next growing season. The importance of this phenomena for nutrients recycling is well documented for decades. On another hand, it isn't clear if the external and internal N levels could modulate the autumnal senescence timing or progression. Consequently, discriminating the autumnal senescence from that of N deficiency- induced senescence type in response to N availability is experimentally confusing and needs a critical concern. Here, we grew hybrid aspen plants (T89) in mull soil that has the minimum amount of nutrients including organic N mainly in organic matters. Group of plants were subjected to modified nutrients solution containing all the needed nutrients including 9mM NH_4NO_3, while another group of plants was subjected to 0mM NH_4NO_3. As expected, the senescence started earlier in older leaves of low N treated plants compared to 9mM NH_4NO_3 treated plants. Hence, the metabolomics profiling was performed of these leaves. Surprisingly, the N deficiency- induced senescence didn't coincide with sugars accumulation as was commonly reported in other plant species. To determine if autumnal senescence is also modulated by N availability, we followed the senescence of the younger leaves (at the middle of the shoot) of the same plants. The senescence of these leaves started on the same date, 25th of September, regardless of the N levels. We studied the N influence on autumnal senescence of tree growing in their natural environment, using a developed system for precise delivering of solutes to the xylem of large aspen trunks. This includes different doses of inorganic N (KNO_3 or NH_4NO_3) or amino acids (Arg, Glu, Gln, or Leu). While the addition of nitrate slowed down the autumnal leaf senescence progression, the addition of the same amounts of amino acids did not.

Key words: Autumn, Senescence, Nitrogen, Aspen, Metabolomics

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

The SnrK1-C/S1 bZIP transcription factor network supports metabolic reprogramming and survival during dark-induced senescence

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Sustaining energy homeostasis is crucial to every living being. Hence, upon energy limiting conditions such as low light exposition or extended darkness (Dark-Induced Senescence, DIS) plants respond with major transcriptional changes as well as metabolic adjustment. To balance energy supply and demand, plants make use of an evolutionarily conserved managing system consisting of two counteracting kinases: TOR (TARGET OF RAPAMYCIN) supports anabolic, energy-consuming metabolism, whereas SnRK1 (SNF1-RELATED PROTEIN KINASE1) activates catabolic, energy-preserving responses. Here, we provide evidence that the nine Arabidopsis group C and S1 bZIP (basic leucine zipper) transcription factors perform as downstream mediators of SnRK1 during DIS (1). We provide a mechanistic model how SnRK1 phosphorylates group C bZIP63, which triggers the formation of C/S1-heterodimers and thus, the recruitment of SnRK1 directly to target promoters (2,3). Precisely, alternative metabolic pathways are activated, which support mitochondrial respiration and hence, enable survival upon starvation. Moreover, particular bZIPs are involved in controlling DIS phenotypes, such as chlorophyll breakdown. Taken together, we propose that the SnRK1-C/S1-bZIP network acts as a major hub orchestrating plant energy homeostasis and survival.

(1) Dröge-Laser and Weiste TIPS (2018), (2) Mair et al. eLIFE (2015), (3) Pedrotti et al. Plant Cell (2018)

Key words: DIS - SnRK1 - transcriptional control - Metabolic adjustment

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

Involvement of oilseed rape PI-WSCPs "Protease Inhibitors - Water Soluble Chlorophyll Binding Proteins" in nitrogen management and stress tolerance

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Winter oilseed rape is a high nitrogen (N)-fertilizer consuming crop characterized by rather low N use efficiency (NUE). In the context of climate change and N inputs regulation, attention is focused on improving NUE under abiotic stress to secure yield. A high proportion of assimilated N remains immobilized in senescent leaves and is returned to the soil failing to contribute to seed yield. Enhancement of nutrient recycling and partitioning performance from senescing tissues to the growing and reproductive organs is likely to improve overall NUE. Effective remobilization of N and C requires a fine tuning between sink demand, associated with a photosynthetic efficiency and source supply related to proteolysis activity. In this context, PI-WSCPs, described as bi-functional proteins acting as both chlorophyll carriers and protease inhibitors, should be involved in maintaining the integrity of the photosynthetic apparatus and controlling the reallocation of proteolytic N. Thus our work aims at investigating PI-WSCPs during leaf development and senescence in relation with N fertilization levels and water availability. PI-WSCP are duplicated genes with 27 copies identified in B. napus genome grouped in structural clusters. Proteins display in silico parietal and vacuolar sub-cellular localizations and exhibit functional divergence between PI activity and chlorophyll binding. The contribution of PI-WSCPs in leaf senescence was investigated on B. napus plants grown under two N regimes and submitted to water stress. PI-WSCPs show sub-functionalization trends related to leaf developmental status and in response to abiotic adverse conditions. Results are discussed in regards to source-sink relationships and N management.

Key words: Nitrogen recycling management, Drought stress, Leaf senescence, Source-sink relationships

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

Role of the RSS1-PP1 pathway in plant tolerance to abiotic stresses

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As sessile organisms, plants are permanently exposed to various environmental constraints such as drought and salinity. To cope with these abiotic stresses, plants evolved complex cellular and molecular mechanisms controling cell division and differentiation in meristematic tissues. RSS1 (Rice Salt Sensitive 1) and Type one protein phosphatase (PP1) are major regulators in the signaling pathway linking salt stress perception to meristem maintenance where RSS1 acts probably by inhibiting PP1 activity during G1/S transition. Our work shows that the wheat RSS1 counterpart TdRL1 (*Triticum durum* RSS-Like 1) is the functional homolog of RSS1 since it was able to complement the salt hypersensitivity of *rss1* mutant. When over-expressed in *Arabidopsis*, TdRL1::GFP was present in dividing cells of the root apical meristem. Further cytological studies show that TdRL1 is cytoplasmic in interphase and associated to the spindle during mitosis. Remarkably, TdRL1 changes its subcellular localization under salt stress with a partial nuclear accumulation, highlighting its multifunctional nature during salt stress response. Moreover, TdRL1 transgenic lines showed increased germination rates under salt stress conditions as compared to wild type. This enhanced salt stress tolerance was associated to an alleviation of oxidative damage. On another hand, co-immunuprecipitation experiments show that TdRL1 is able to interact with a wheat PP1 (TdPP1a) and to dampen its phosphatase activity. In BiFc assays TdPP1a and TdRL1 co-localize within cortical microtubule network. *Arabidopsis* lines over-expressing TdPP1a::GFP showed enhanced abiotic stress tolerance and increased root growth under brassinosteroid (BR) treatments. Interestingly, TdPP1a seems to interfere with BR signalling via its interaction with BES1 (a transcription factor that regulates the expression of BR-responsive genes) and increasing its dephosphorylated (active) level. The questions regarding the contribution of the RSS1-PP1 pathway in the maintenance of meristems under stress conditions and its connection with the BR signaling in enhancing plant stress tolerance are currently investigated.

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

BEP - Barley Epigenome Project

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Stress-induced, premature leaf senescence in crop plants severely impairs yield and causes massive economic loss. To cope with this problem, we need to understand the underlying molecular mechanisms. Recent research revealed that stress responses and leaf senescence are controlled by higher order epigenetic mechanisms. Our innovative goal is to establish at the Science Campus Halle an epigenome platform for crop plants which enables genome wide analyses of epigenetic histone and DNA modifications. Using this platform, in a first step we want to identify the epigenetic key regulators of leaf senescence with the aid of qRT-PCR, Chromatin immunoprecipitation followed by deep sequencing and transcriptome analysis by RNA-Seq. This will be very important for targeted breeding approaches aiming at crops which are more tolerant to stress.

Key words: Epigenetics, Barley, Drought stress, Histone modifications, Sequencing

SESSION 3: STRESS-INDUCED SENESCENCE

Oral presentation

Transcriptomics of stress induced and development dependent senescence in barley

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Senescence is the last step of leaf development and finally results in leaf death. The process of leaf senescence is characterized by drastic reprogramming of gene expression, during which some genes are upregulated (SAGs – senescence associated genes), while others are downregulated (SDGs – senescence downregulated genes). Within the leaf, these alterations lead to various molecular and structural changes, like decrease of chlorophyll content, reduced photosynthetic activity and also to the recycling of resources.

During development dependent senescence an efficient recycling program provides high quality and quantity especially in annual crop plants. This can be ensured, inter alia, by strictly regulating the reprogramming of gene expression at various levels. Due to their sessile lifestyle, plants are exposed to an ever-changing environment, including a wide variety of environmental conditions. Premature, via stress, induced senescence processes are known to reduce agricultural productivity. Beside various biotic and abiotic stressors, a reduced availability of water is of central importance.

As the knowledge of the regulatory network during senescence is still fragmentary, the aim is to identify central regulatory factors of different regulatory levels, which connect development dependent and stress induced leaf senescence. Therefore, we grew barley plants under drought stress and well-watered conditions and characterized drought stress induced and development dependent senescence of barley primary leaves on physiological and molecular level. Rewatering of drought stressed plants could delay the senescence syndrome.

Global changes of gene expression under drought stress conditions are compared via Microarray Analysis with developmental senescence. A set of transcription factors, epigenetic factors with an interesting co- or counter- regulation under drought stress induced and development dependent senescence could be identified. As small RNAs (have a strong influence on the regulation of gene expression on transcriptional and posttranscriptional level, 159 pri-miRNAs were tested with RT-qPCR platform to identify potential senescence regulating miRNAs in barley.

Key words: Drought stress, *Hordeum vulgare*, MiRNA, Regulation of gene expression, Transcriptomics

SESSION 3: STRESS-INDUCED SENESCENCE

Poster presentation C1

Arabidopsis NITROGEN LIMITATION ADAPTATION regulates ORE1 homeostasis during senescence induced by nitrogen deficiency

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Nitrogen is an important macronutrient in plants and its deficiency induces rapid leaf senescence. Two genes, ORE1 and NITROGEN LIMITATION ADAPTATION (NLA), have been implicated in regulating the senescence process but their relationship is unclear. Here, we show that nla and pho2 (also known as ubc24) plants develop rapid leaf senescence under nitrogen-starvation condition, whereas ore1 and nla/ore1 and pho2 (ubc24)/ore1 plants stay green. These results suggest that ORE1 acts downstream of NLA and PHO2 (UBC24). NLA interacts with ORE1 in the nucleus and regulates its stability through polyubiquitination using PHO2 (UBC24) as the E2 conjugase. Our findings identified ORE1 as a downstream target of NLA/PHO2 (UBC24) and showed that post-translational regulation of ORE1 levels determines leaf senescence during nitrogen deficiency.

Key words: Nitrogen deficiency, Ubiquitination, NLA, ORE1

SESSION 3: STRESS-INDUCED SENESCENCE

Poster presentation C2

Senescence-Associated Ethylene Responsive Factor 1 (SAERF1) functions as a molecular linker between leaf senescence and salt stress response in Arabidopsis

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Leaf senescence is a complex developmental process modulated by a repertoire of endogenous and external factors. Many studies have been dedicated to investigating how plants coordinately regulate leaf senescence in response to developmental programs and environmental stresses. However, our knowledge of the intricate regulatory mechanisms that integrates signals from environmental factors into intrinsic age-mediated programs is still fragmentary. Here, we identified and characterized Senescence-Associated Ethylene Responsive Factor 1 (SAERF1), a ERF family transcription factor in Arabidopsis whose expression is differentially changed during leaf senescence triggered by age as well as diverse environmental stresses. SAERF1 played a negative role in regulating leaf senescence induced by senescence-inducing factors such as age, dark, abscisic acid, oxidative stress, and salt stress. Furthermore, we demonstrated that SAERF1 conferred salt stress tolerance during various developmental stages including seed germination, seedling growth, and vegetative growth stages. SAERF1 was capable of strongly activating the transcription of the reporters in yeast, indicating the potential action of SAERF1 as a transcription activator. Through the identification of genome-wide direct targets of SAERF1, we also revealed that SAERF1 directly bound to the promoters of several salt stress-responsive genes including RD22 and RD29a and activated their expression to negatively regulate salt stress-induced leaf senescence. The latest progresses on the molecular conjunction between leaf development and salt stress response through SAERF1 will be further presented.

Key words: ERF, Salt stress, Arabidopsis, Leaf senescence, Transcription factor

SESSION 3: STRESS-INDUCED SENESCENCE

Poster presentation C3

Identification of the regulatory mechanism through which JUB1 confers tolerance to drought

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The NAC transcription factor (TF) JUNGBRUNNEN1 (JUB1) is a central negative regulator of plants senescence in Arabidopsis. JUB1 strongly delays senescence when overexpressed in transgenic plants and triggers precocious senescence at the low expression level in the jub1 knockdown mutant. Interestingly, several groups of transcription factors that have been reported to play a significant role in the regulation of senescence also have been shown to be involved in plant stress tolerance. We also reported that in addition to promoting juvenility overexpression of *JUB1* leads to enhanced tolerance to abiotic stress. Understanding the mechanisms behind these
processes can provide effective strategies for the generation of plant stress tolerance. Evidence from our lab indicates that a higher level of proline, regulation of the stomatal aperture/development, or scavenging of ROS via direct regulation of *DREB2A* might be the cause for the enhanced tolerance of plants overexpressing *JUB1* to drought stress. Our data show that *JUB1-OX* plants accumulate proline under control conditions at the early and later stages of development. Plants overexpressing *JUB1* also show a higher accumulation of proline during the onset of drought and at the later stages of drought. We found that overexpression of *JUB1* induces proline biosynthesis genes and suppresses proline degradation genes. In addition, our preliminary data indicate that *JUB1-OX* plants exhibit lower stomatal conductance under control and drought conditions. Our results suggest that JUB1 is a promising target for future work to improve the tolerance of plants against abiotic stresses.

Key words: Stress tolerance, Transcription factors, Gene regulatory Networks, Proline metabolism, Stomatal conductance

SESSION 3: STRESS-INDUCED SENESCENCE

Poster presentation C4

NAC Transcription Factor SlST1 Negatively Regulates Salt Induced Senescence in Tomato

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Salinity stress negatively affects growth and yield in many plants including vegetable crops and thus has an enormous impact on agricultural productivity. Here, we identified a novel NAC transcription factor SlST1 (*Solanum lycopersicum* Salt Tolerant 1) as a negative regulator of salt-induced senescence in tomato. Expression of SlST1 is strongly induced by salinity, H_2O_2 and the stress-responsive phytohormone abscisic acid (ABA). Transgenic tomato plants moderately overexpressing SlST1 from its own promoter revealed a reduction in salt-induced senescence, accompanied with higher leaf chlorophyll content, reduced expression of senescence-associated genes SAG13 and SAG15, and a decline in electrolyte leakage (an indicator for membrane damage). By contrast, the effect of salt-induced senescence was much more pronounced in SlST1 knockdown and CRISPR-Cas9-generated knockout lines. Under salt stress, SlST1-knockdown plants accumulated higher levels of harmful Na+ ions in leaves compared to wild type, while SlST1 moderate overexpressors accumulated less Na+ ions, in accordance with the altered expression of the Na+ transporter genes SlHKT1;1 and SlHKT1;2. On the other hand, the compatible osmolyte proline was more abundant in SlST1 overexpressors, but less so in SlST1-knockdown lines under salt stress in accordance with the reduced and elevated salt-induced senescence, respectively, of these lines. Furthermore, we found that SlST1 regulates salt stress-responsive genes and binds to their promoters in vitro, identifying them as potential direct target genes of the SlST1 transcription factor. Finally, we conclude that SlST1 plays an important role in the regulation of the response to salt stress and this transcription factor can be utilized for an agronomic perspective to enhance the yield of tomato plants during salinity stress.

Key words: Transcription factors, SlST1, Salt stress-induced senescence, proline, Ion homeostasis

SESSION 3: STRESS-INDUCED SENESCENCE

Poster presentation C5

Overexpression of Type one protein phosphatase and their involvement in Brassinosteroid signalling and abiotic stress

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Abiotic stresses such as drought and salinity induce the process of senescence and result in significant reduction in crop yields. Improving salt tolerance by avoiding or delaying senescence under stress will therefore play an important role in maintaining high agricultural productivity. To overcome these environmental constraints, plant evolved various complex cellular and molecular mechanisms that are mainly driven by reversible phosphorylation. Type one protein phosphatases participate in biological processes such as cell cycle regulation, development, blue light-dependent stomatal opening, signaling of hormonal pathways, and salinity tolerance. Interested in maintaining crop productivity under adverse conditions, we focused on the understanding of wheat PP1's role in plant responses to environmental cues. Therefore, we cloned *TdPP1*a, the first wheat type one protein phosphatase from a Tunisian durum wheat variety. TdPP1a is highly conserved in sequence and structure when compared to other plant PP1s and is functional *in vitro* and *in vivo*. We generated three independent homozygous lines of *Arabidopsis thaliana* overexpressing TdPP1a::GFP which exhibited enhanced abiotic stress tolerance. Furthermore, we observed in our transgenic lines exposed to brassinolide, an enhanced root growth compared to wild type seedlings grown in the same conditions. Further experiments showed that TdPP1a was able to interact with BES1, a transcription factor regulating Brassinosteroid responses. The level of dephosphorylated and active BES1 was increased in the transgenic lines. Transcriptional analyses revealed the modification of BES1-regulated genes upon PP1 over-expression indicating a positive regulation of BR signaling in our transgenic lines. These novel and interesting data suggest a positive role of TdPP1a in plant stress responses probably through modulation of brassinosteroid signaling.

Key words: Durum wheat, Type 1 protein phosphatase, Abiotic stress, Brassinosteroids, BES1.

SESSION 3: STRESS-INDUCED SENESCENCE

Poster presentation C6

Exogenous application of hydrogen sulfide to maize (*Zea mays* L) seedlings confers tolerance to chromium by suppressing peroxynitrite derived stress and H_2O_2 detoxification

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Chromium (Cr) contamination is a concern for sustainable agricultural production and food safety. Several studies have developed new alternatives to chemical inputs, such as the artificial application of signal molecules through their donors (precursors). Hydrogen sulphide (H_2S) and nitric oxide (NO) are considered as a signaling gas emitter involved in the modulation of physiological processes in animals and plants. The effects of NaHS (sodium hydrosulfide) and HA (hydroxylamine), potential donors and biosynthetic inhibitor of H_2S, on heavy metal stress mitigation were investigated in maize (*Zea mays* L.) exposed to Cr (VI) during germination. Analysis of Cr content, oxidative stress indicators : H_2O_2, protein carbonylation, proline and methylglyoxal (MG) accumulation revealed that Cr causes oxidative stress. However, supplementary treatment of heavy metal-exposed seedlings by NaHS allowed all these parameters to be restored to the control. Moreover, the addition of N(ω)-nitro-L-arginine methyl ester ($_L$-NAME; an NO biosynthesis inhibitor) reduced the protective effect of H_2S against Cr. An important interest is whether NO and H_2S are inter-related and therefore inter-regulated, as these two metabolites and their derivatives can react with each other. These results are corroborated by those resulting from the evaluation of a potential oxido-nitrosative events focusing peroxynitrite derived stress, polyamine status and ascorbate-glutathione cycle behavior. Chromium-induced peroxynitrite ($^{-}$ONOO) production was directly related to the high accumulation of $O_2\bullet^-$, while in the presence of NaHS low cellular contents of $O_2\bullet^-$ limit the generation of $^{-}$ONOO. The stimulation of NO production and the increase in the endogenous content of H_2S cause the nitrosylation of GSH whose recycling with ascorbate suggests a modulation of the activities of enzymes of the ascorbate-glutathione cycle by the S-nitrosylation signal.

Key words: Chromium, Hydrogen sulfide, Maize, Nitric oxide, Methylglyoxal, Oxido-nitrosative stress, Polyamines

Session 4

TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

Systems biology of leaf ontogenesis in tobacco – from thylakoid biogenesis to senescence

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The composition of the photosynthetic apparatus of higher plants is dynamically adjusted to long-term changes in the metabolic demand for ATP and NADPH imposed by stresses and leaf development. By changing photosynthetic complex stoichiometry, an imbalance between the photosynthetic production of ATP and NADPH and their metabolic consumption is avoided, and cytotoxic side reactions are minimized. We systematically compared the acclimation capacity of developing, mature and senescing leaves of tobacco to changing environmental conditions. Different to young developing leaves, the capacity of mature and senescing leaves to acclimate is very limited, because their photosynthetic complex biogenesis is massively repressed. This is possible because the cytochrome b6f complex, photosystem I and chloroplast ATP synthase are highly stable. They are only synthesized in young leaves, and under most conditions, their biogenesis is inactivated once the photosynthetic apparatus is fully established. Also strong reductions in chloroplast ribosome abundance restrict complex biogenesis in mature leaves. The residual ribosomes are largely required to sustain the repair of photosystem II, which has a much higher turnover than the other photosynthetic complexes. To gain more insights into the molecular basis of the repressed photosynthetic complex biogenesis in mature and senescing leaves, we performed a system-biology analysis of changes in photosynthetic complex accumulation, electron transport and leaf assimilation, chloroplast structure, and gene expression in both nucleus and chloroplast during the entire leaf ontogenesis of tobacco. Also metabolite profiles were generated. These data now enable us to gain detailed insights not only into the mechanisms controlling complex accumulation during leaf ontogenesis, but also to determine the underlying cellular and systemic signals.

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

Cell separation as a final stage of flower senescence: Novelties and challenges in controlling floral abscission

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Abscission of flowers and floral organs is an important developmental process, which represents the final stage of flower senescence. The abscission process, which occurs in a layer of functionally specialized cells - the abscission zone (AZ), is regulated by endogenous and exogenous signals, and involves the activity of cell wall degrading enzymes and secretary pathways. Recent reports, new methods, and novel insights revealed several new aspects of floral abscission, which encourage future research efforts. A) Demonstration that a specific and gradual increase in the cytosolic pH of AZ cells occurs during natural abscission of flower organs, and coincides with the execution of floral organ abscission in Arabidopsis, wild rocket, and tomato. The future challenge is to track the pH changes in the apoplast and cell wall, in which the enzymes required for cell separation are activated. B) Analysis for the first time of the expression kinetics of genes encoding the components involved in vesicle trafficking in the tomato flower AZ, by using a tomato AZ-specific microarray chip. The results obtained clearly demonstrate how the processes of protein secretion by vesicle trafficking are regulated, programmed, and orchestrated at the level of gene expression. These data provide target proteins to design appropriate antibodies for affinity purification of plant vesicles in their natural state. This will enable to analyze and dissect the vesicle trafficking networks for further understanding of flower abscission. C) A conclusion based on available data confirms that ethylene is the key regulator of plant organ abscission, whereas the pathway of INFLORESCENCE DEFICIENT IN ABSCISSION-HAESA-HAESA-like2 (IDA-HAE-HSL2) acts downstream of ethylene signaling. It is also suggested that the ability of an organ to abscise is tightly linked to cell turgidity in the AZ. This hypothesis and the physiological role of the IDA-HAE-HSL2 pathway in cell separation remain as open challenges for exploration.

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

Characterization of plant age-dependent root senescence in barley

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Aging-related processes in roots are associated with a decrease in root functions that are relevant for stress tolerance and plant performance. We tested the hypothesis whether aging-related processes in roots comply with typical features of senescence. Morphological, physiological and molecular changes in root traits of the seminal root system in hydroponically-grown barley plants were monitored over a period of six weeks. Arrested root elongation, lysis of the root cortex, and decreases in nitrate uptake rate as well as in auxin and cytokinin concentrations coincided at the same time point, i.e. 39 days after germination. At this time point, root abscisic acid (ABA) levels peaked, suggesting that ABA triggers root aging-related processes. Transcriptome profiling revealed upregulation of several NAC-, WRKY- and AP2-type transcription factors in apical and in basal root tissues, partly coinciding with the induction of genes involved in proteolysis and oxidative stress responses. The present study provides a first comprehensive framework for the chronological sequence of root processes during aging and shows that seminal roots undergo changes that are highly reminiscent of leaf senescence and subject to a genetically-determined senescence program that is under predominant influence of plant age

Key words: Aging, Root type, Transcriptome, Phytohormones, Nutrients, Metabolism

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

A suppressor of axillary meristem maturation promotes longevity in flowering plants

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My lab has a long-standing history in studying the central role of the hormone auxin in plant development, with a specific focus on its polar transport and how abiotic signals alter plant development by changing the direction of this transport. More recently, my research interests have expanded to the role of auxin in plant developmental transitions or phase changes, such as the initiation of (somatic) embryogenesis and fruit development, and how these transitions mediate plant ageing.

In flowering plants, ageing is defined by a series of developmental transitions that starts with vegetative growth, and is followed by flowering, culminating in seed production. Tissue senescence and plant death follow seed production in monocarpic plants, while polycarpic plants prolong their life span by maintaining a number of vegetative axillary meristems, thereby allowing subsequent cycles of vegetative and reproductive development.

We identified a suppressor of axillary meristem maturation in *Arabidopsis thaliana*, with effects on plant shoot architecture and -longevity. Loss of suppressor function accelerated plant aging, whereas overexpression maintained vegetative axillary meristems and converted monocarpic *A. thaliana* and *Nicotiana tabacum* into (polycarpic) plants with reduced senescence, a prolonged life span and enhanced seed and biomass production. The position of this suppressor in the plant ageing pathway will be presented and discussed.

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

KIRA1 and ORESARA1 terminate flower receptivity by promoting senescence-induced programmed cell death in the Arabidopsis stigma

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Flowers have a species-specific functional life span that determines the time frame in which pollination, fertilization, and seed set can successfully occur. The tissue of the floral stigma plays a key role in flower receptivity serving to intercept pollen and initiate pollen tube growth towards the ovary. Here we show that a tightly controlled age-induced programmed cell death process terminates the functional life span of the non-pollinated stigmatic papilla cells in *Arabidopsis thaliana*. We identified the leaf senescence regulator ORESARA1/ANAC092 and the previously uncharacterized KIRA1/ANAC074 as partially redundant key transcription factors that regulate stigma life span by directly controlling the expression of cell-death associated genes. *KIRA1* expression is sufficient to induce programmed cell death and effectively terminate floral receptivity, while lack of both *KIRA1* and *ORESARA1* substantially increases stigma life span. Surprisingly, however, the extension of stigma life span causes only a moderate extension of flower receptivity, suggesting that next to the stigma cell death program, other stigma-expressed pathways participate in the control of the flower's receptive life span.

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

Stem cell ageing in the *Arabidopsis thaliana* root

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Plants form new organs from pluripotent stem cells throughout their lives and under changing environmental situations. In Arabidopsis root meristems, tissue specific stem cells are maintained through direct contact with an organizing centre, which is named quiescent centre (QC) because of its relative mitotic inactiveness. Distally from the QC lie the columella stem cells that give rise to the columella cells, which function in gravity sensing and are constantly shed off to enable the root tip to growth through the soil (Dolan et al., 1993). This three-layered stem cell system of QC, columella stem cells and differentiated columella cells serves as a model for stem cell research because of its stereotypic and relatively simple organization. The regulatory molecular network responsible for maintenance of root stem cells has been investigated thoroughly in the past years. One key regulator of the root apical meristem is the QC expressed WOX5 (WUSCHEL-RELATED HOMEOBOX 5) transcription factor (Sarkar et al., 2007). It travels from the QC to the columella stem cells to epigenetically maintain their undifferentiated state (Pi et al., 2015). Previous studies predominantly used roots up to seven days after germination. However, in aging roots, the root apical meristem displays a gradual loss of the stereotypic patterning and an increasing division rate of the QC (Baum et al., 2002). This implies that the genetic network of stem cell homeostasis changes with age. In this work, we investigate the morphological and molecular parameters of columella stem cell ageing.

Key words: *Arabidopsis thaliana*, Root apical meristem, Columella stem cells, Ageing, WOX5

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Oral presentation

Characterization of stem senescence of annual and perennial Brassicaceae species

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Flowering shoots eventually undergo senescence as seed maturation progresses. This phenomenon can be observed in plants flowering only once (annuals) as well as in plants flowering repeatedly over several years (perennials). We studied the progress of shoot senescence from opening of the first flower onwards in several Brassicaceae species including annual *Arabidopsis thaliana* as well as the perennial Arabis alpina. As expected only the inflorescence stem undergoes senescence in the perennial, whereas the annual showed whole plant senescence. So far stem senescence is rarely studied and it is unknown whether a molecular program exists.

In order to get insight into the regulation of stem senescence we performed morphological analyses of the inflorescence and vegetative stem, which exhibited either primary or secondary growth, respectively. However, due to secondary growth in Arabis the outer cell layers died and a new epidermis was formed, which was accompanied by starch accumulation. RNA sequencing revealed major transcriptional changes of up to 30% of the genome 28 days after flowering of the inflorescence stem in both species, as well as 49 days after flowering in the vegetative stem of Arabidopsis. Detailed analysis revealed that genes known to play a role in programmed organ senescence are upregulated in inflorescence stem as well as Arabidopsis vegetative stem, e.g. ORE1, ANAC046, NAP, NYC1 and SAG12. These genes were also confirmed to be upregulated during leaf senescence in both species, which might indicate a conserved role of these genes in Arabis and also general inflorescence senescence. The RNA sequencing also might imply that inflorescence as well as whole plant senescence in monocarpic species might be a controlled program rather than a degenerative uncontrolled process. Further studies using transgenic lines of Arabidopsis and Arabis will help to gain more insight into the spatio-temporal control of stem senescence in annuals and perennials.

Key words: Stem senescence, Comparative transcriptome analysis, Annual, perennial, *Brassicaceae*

Session 4: TISSUE- AND ORGAN-SPECIFIC ASPECTS OF SENESCENCE

Poster presentation D1

Hormonal signals determining yield potential in barley

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Barley (*Hordeum vulgare* L.) possesses an unbranched 'spike' type inflorescence with determinate floret-bearing spikelet triplets attached to its inflorescence axis in a distichous pattern. The indeterminate inflorescence meristem (IM) of barley produces spikelets continuously along its axis until reaching its maximum yield potential (MYP) stage. After this stage, there is a gradual decrease in the spikelet numbers due to the death of spikelets starting from the apex to a certain position in spike. Percentage of this pre-anthesis spikelet decline varies among genotypes and has an ultimate impact on final grain number. Until now, there have been no molecular studies to understand this pre-anthesis developmental program. In the present study, we investigated the involvement of endogenous hormones in the process of spikelet decline in the two-rowed barley cv. Bowman by measuring several hormones in different spike sections at early developing stages. After the stamen primordium stage (W3.5), there is a steady increase in ABA levels in the apical part of the spike. Between MYP stage (W5.0) and the visible abortion progression stage (W8.0), we found ~ 11-fold increase in ABA only in the aborting, apical regions of the spike; whereas low amounts were maintained in central and basal sections. Along with ABA, its catabolic product phaseic acid also showed a similar distribution pattern. In contrast, growth promoting hormones including IAA, iP, GA19, GA53, GA44 showed lower amounts in aborting tissues compared to well growing central and basal parts. Interestingly, lower JA levels and higher SA levels were similarly found in the aborting region. Our preliminary results suggest that obtained hormonal gradients during spike development have a major impact on spikelet abortion. Whether an increase in ABA is the cause or consequence remains unknown. Further metabolic and transcriptomic studies will help us in a better understanding of this process.

Key words: Barley, Meristem, Spikelet abortion, ABA

Session 5

AUTOPHAGY AND SENESCENCE

Session 5: AUTOPHAGY AND SENESCENCE

Oral presentation

AUTOPHAGY, THE MASTER OF BULK AND SELECTIVE RECYCLING

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Autophagy-mediated turnover plays an essential role in cellular homeostasis by removing damaged organelles and unwanted cytoplasmic constituents, and is critical for plant defense and robust nutrient recycling, especially during nitrogen and fixed-carbon starvation and senescence. This 'self eating' is mediated by a conjugation system that modifies a pair of ubiquitin-fold proteins ATG12 and ATG8 to eventually form an autophagic vesicle coated with the ATG8-phosphatidylethanolamine (PE) adduct. ATG8-PE serves two purposes, one is to help shape the encapsulating vesicles and their subsequent fusion with the vacuole, and the other is to provide a docking platform for a suite of ATG8-interacting proteins that selectively tether appropriate cargo to the membrane surface before enclosure. In addition to bulk degradation, the ATG8 system is responsible for clearing organelles like mitochondria (mitophagy), chloroplasts (chlorophagy) and peroxisomes (pexophagy), and large cytoplasmic complexes such as 26S proteasomes (proteaphagy) or ribosomes (ribophagy) when dysfunctional or no longer needed. Using a multi-omics approach with maize, we are attempting to understand how autophagy regulates the metabolome and sculpts the proteome during normal growth and during nutrient starvation. Surprisingly, broad alterations in the leaf metabolome were evident in plants missing the core autophagy component ATG12 even without stress, particularly affecting products of lipid turnover and secondary metabolites, which were underpinned by substantial changes in the transcriptome and/or proteome. Cross-comparison of mRNA and protein abundances allowed for the identification of organelles, protein complexes, and individual proteins targeted for selective autophagic clearance, and revealed several processes controlled by this catabolism. During our work selective proteaphagy, we discovered that ubiquitylation of dysfunctional complexes followed by their recognition by the autophagy receptor RPN10 are key to this clearance. Surprisingly, further studies on RPN10 revealed that it represents the founding member of a new class of autophagy adaptors/receptors that uses a UIM instead of an AIM sequence for binding to ATG8. Assays with candidate UIM proteins and non-biased screens revealed that these adaptors/receptors are likely present in all eukaryotes. One family of UBX-UIM proteins are of particular interest as they help direct the degradation of the AAA-ATPase CDC48/p97 hexamer that couples ATP hydrolysis to the extraction and removal of damaged proteins associated with ER stress. With this new class of adaptors/receptors, we greatly extend the reach of selective autophagy and potentially identify new factors regulating autophagic vesicle dynamics.

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Session 5: AUTOPHAGY AND SENESCENCE

Oral presentation

Autophagy and Nutrient Remobilization during Senescence

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Plants have the advantage of being able to produce their energy source by fixing carbon from the air through photosynthesis. However, under some conditions, such as during the development of non-photosynthetic tissues, senescence or abiotic stress, in which photosynthesis is downregulated, there is a shortage in carbon supply. Therefore, other resources must be used to meet the plant's energy demands. Nutrient supply is thus, at least partially, met by the degradation of cellular components, resulting in pronounced metabolic changes.

Autophagy is a conserved eukaryotic process for the degradation of cellular constituents in the lytic compartment (vacuole in yeast and plants and lysosome in animals). The targets of autophagy are diverse and include soluble proteins, protein aggregates, whole organelles, and lipids. The autophagy mechanism is highly conserved, and homologs of many autophagy-related (ATG) genes have been characterized in plants, including the model plant *Arabidopsis thaliana* and the crop plant tomato (*Solanum lycopersicum*). The hallmark phenotype of autophagy-related mutants (*atg* mutants) is higher sensitivity nutrient starvation, early senescence, and lower yield. However, the direct impact of autophagy on cellular metabolism has not been well studied.

Our group studies nutrient remobilization in plants, focusing on autophagy as a model system for this process, by investigating the role of autophagy during different stages in plant life in which nutrient remobilization is crucial. We specifically focus on carbon starvation and fruit development in Arabidopsis and tomato, both accompanied by senescence. We observed distinct morphological differences between WT and *atg* mutant plants suggesting delayed growth and early senescence. We employed high-throughput metabolomic, lipidomic and proteomic analyses as well as extensive flux analyses, in order to elucidate the underlying causes of the morphological phenotype. We were able to show that autophagy has a global effect on central metabolism under conditions in which nutrient remobilization is necessary.

Session 5: AUTOPHAGY AND SENESCENCE

Oral presentation

Autophagy and plant proteases for N remobilization during leaf senescence

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Senescence is a long developmental process dedicated to the shift from primary nitrogen and carbon assimilation to progressive cell-constituent degradation and catabolism for nutrient recycling and remobilization at the whole plant level. Autophagy is enhanced during developmental leaf senescence and even more severely when nutrient limitation is applied. Autophagy mutants are strongly impaired in the remobilization of nitrogen from the rosettes to the seeds, especially when cultivated under low nitrate conditions. Autophagy machinery involves up to 40 ATG proteins in Arabidopsis. The overexpression of *ATG8* in Arabidopsis increases autophagic activity. *ATG8* overexpressors display higher N remobilization efficiency but only when nitrate supply is plethoric.
Autophagy mutant that cannot recycle and remobilize nitrogen accumulate numerous protease activities amongst which several well-known cysteine proteases. The proteases accumulated in autophagy mutants may compensate for weaknesses of mutants in N-recycling and play as alternative degradation pathway regarding autophagy-dependent degradation. Their role in N remobilization is under investigation and links to the global proteome disorders observed in autophagy mutant are under study.

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Key words: Autophagy, Protease, Remobilization, Senescence

Session 5: AUTOPHAGY AND SENESCENCE

Oral presentation

Multiple omics uncover the roles of autophagy on maintaining the balance of endomembrane compositions in Arabidopsis

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Large scale proteomic analysis was preformed to identify the proteins that significant expressed in the comparisons of both atg5 vs. wild-type and atg5/sid2 vs. sid2 under various N and S nutritive conditions. Salicylic-acid independent effects were determined by comparing the autophagy responsive proteins from wild-type and sid2 background. The data revealed that mutation in atg5 triggered the endoplasmic reticulum (ER) stress, and increased the peroxisome and ER proteins involved in very long chain fatty acid synthesis and β-oxidation. The contents of sphingolipid, phospholipid and galactolipid were significantly modified. Changes in the length of fatty acid chains confirmed that the ER lipid-metabolism was modified in atg5. Significant accumulations of phospholipids and ceramides and changes in GIPCs (glycosyl-inositol-phosphoryl-ceramides) indicated that atg5 mutants suffer from large modifications in endomembrane -and especially plasma membrane-lipid composition. Decreases in chloroplast proteins and galactolipids in atg5 was mostly restricted to low nutrient conditions, and suggested that under starvation especially, chloroplasts were used as lipid reservoirs for β-oxidation in atg5 mutants. These data demonstrates the roles of autophagy on the regulation of ER stress and reveals the role of autophagy in the control of plant lipid metabolism and catabolism, influencing both lipid homeostasis and endomembrane composition.

Key words: Macroautophagy, Metabolism, Peroxisomes, Plant autophagy, Plants

Session 5: AUTOPHAGY AND SENESCENCE

Oral presentation

Selective autophagy regulates thermomemory in *Arabidopsis thaliana*

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Heat stress (HS) is one of the major threats to plant growth and productivity, and it strongly induces precocious senescence. Experimental evidence suggests that plants have a memory for HS (thermomemory). Pre-exposure of plants to high, but non-lethal temperature (thermopriming) enables them to perform more efficiently upon future, severe HS through the acquisition of memory (formation and maintenance of the acclimated state). However, the molecular mechanisms underlying the formation, duration, and resetting of stress memories remain largely unexplored. Recently, our lab identified a novel role of autophagy for the regulation of thermomemory in *Arabidopsis thaliana*. Autophagy is an evolutionarily conserved and dynamic recycling process critical for maintaining cellular energy homeostasis. While it has been considered that autophagy is a bulk degradation process, recent advances provide evidence that autophagy can be highly specific. Here, by non-targeted proteomics, we identified NBR1 (Neighbor of BRCA1), a receptor for selective autophagy, as a regulator of thermomemory in Arabidopsis. Mutants lacking *NBR1* revealed a better thermomemory than Col-0. Immunoblot analysis showed a higher accumulation of NBR1 protein during the thermomemory phase in autophagy-deficient mutants than in Col-0. Using confocal microscopy, we demonstrated an accumulation of NBR1 bodies and an enhanced activity of NBR1 during the thermomemory phase. Furthermore, we show an involvement of autophagy in the degradation of NBR1. To validate the role of NBR1 during thermomemory, we carried out shotgun proteomics on the *nbr1-2* mutant compared to Col-0 and identified novel substrates of NBR1. Moreover, we validated the involvement of NBR1 in degrading its cargo proteins during the thermomemory phase. Our study highlights the novel and crucial role of NBR1 for selective degradation of memory-associated proteins by autophagy and could provide a molecular link between heat stress and senescence in Arabidopsis.

Key words: *Arabidopsis thaliana*, Autophagy, Thermomemory, Proteomics, Senescence

Session 5: AUTOPHAGY AND SENESCENCE

Poster presentation E1

APG transcription factor regulates senescence and autophagy in *Arabidopsis*

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Autophagy is a catabolic process involved in degrading and recycling cellular components. The regulation of autophagy occurs at different levels. However, the transcriptional regulation of autophagy remains mostly unknown. The endoplasmic reticulum (ER) is involved in protein synthesis, folding and intracellular trafficking. Disruption in ER homeostasis results in a stress response by exhibiting precocious senescence.

Recently, we identified APG (Autophagy positive regulator), a NAC transcription factor as a novel positive transcriptional regulator of autophagy. Transgenic plants that overexpress APG have higher expression of autophagy-related genes (ATG). Through chromatin immunoprecipitation coupled with quantitative PCR (ChIP-qPCR) and electromobility shift assay (EMSA) we confirmed the direct binding of APG to the promoters of ATG genes in Arabidopsis. Using confocal microscopy, we observed higher numbers of autophagosomes in GFP-ATG8a/APGOX under control conditions. ER stress induces autophagy partially through transcriptional regulation. Consequently, variation in the expression level of APG contributes to the ER stress response and apg mutants displayed an early senescence phenotype upon ER stress. Chemical inducers of ER stress resulted in fewer mature autophagosomes in apgKO/GFP-ATG8a transgenic plants compared to GFP-ATG8a.

Abiotic stress, ABA and chemical inhibitors of Target of Rapamycin induce the expression of APG. The APG promoter harbors three ABA-responsive elements, and in yeast-one-hybrid assay, the APG promoter interacts with the ABRE-binding factors, ABF2, ABF3 and ABF4. In this manner, APG appears to integrate ABA-signaling pathways and respond to abiotic stresses such as ER stress to regulate autophagy and plant senescence.

Key words: Autophagy, Senescence, ER stress, ABA, Transcription factors

Session 6

RELATED TALKS

Session 6: RELATED TALKS

Oral presentation

From genome to pan-genome in barley and wheat

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Genomics based-breeding and research in molecular genetics and developmental biology was lagging behind in barley and wheat, two major crops in Europe and the world, because high quality genome sequences were missing. This has changed now, as for both species annotated high-quality reference chromosome-scale sequence assemblies were made available recently. In both cases the first reference sequence assemblies are representing a big leap forward, however, these assemblies are by far not representative for all domesticated genome diversity in both species. Thus in order to efficiently unlock genomic diversity for research and breeding, international efforts were initiated to describe the pan-genomes of barley and bread wheat by producing multiple high quality chromosome-scale assemblies. In addition, entire diversity collections of wheat and barley comprising several ten thousands of accessions are currently being genotyped by sequence-based methods. The presentation will report on the status of this research in the small grain cereals and provide detailed insights into the level of genome diversity of these major crop species.

Session 6: RELATED TALKS

Oral presentation

Chlorophyll catabolism precedes changes in chloroplast structure and proteome during leaf senescence

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The earliest visual changes of leaf senescence occur in the chloroplast as chlorophyll is degraded and photosynthesis declines. Yet, a comprehensive understanding of the sequence of catabolic events occurring in chloroplasts during natural leaf senescence is still missing. Here, we combined confocal and electron microscopy together with proteomics and biochemistry to follow structural and molecular changes during Arabidopsis leaf senescence. We observed that initiation of chlorophyll catabolism precedes other breakdown processes. Chloroplast size, stacking of thylakoids and efficiency of PSII remain stable until late stages of senescence, whereas the number and size of plastoglobules increase. Unlike catabolic enzymes, whose level increase, the level of most proteins decrease during senescence, and chloroplast proteins are overrepresented among these. However, the rate of their disappearance is variable, mostly uncoordinated and independent of their inherent stability during earlier developmental stages. Unexpectedly, degradation of chlorophyll-binding proteins lags behind chlorophyll catabolism. Autophagy and vacuole proteins are retained at relatively high levels, highlighting the role of extra-plastidic degradation processes especially in late stages of senescence. The observation that chlorophyll catabolism precedes all other catabolic events suggests that this process enables or signals further catabolic processes in chloroplasts and the entire senescing cells.

Session 6: RELATED TALKS

Poster presentation F1

Genomewide identification of *NAC* transcription factor genes in *Chenopodium quinoa*

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Chenopodium quinoa (quinoa) is pseudocereal that has a high nutritional value and has a relatively high tolerance to several abiotic stresses, including drought and salinity, making it a good plant for the study of mechanisms of abiotic stress tolerance. NAC (NAM, ATAF and CUC) transcription factors are involved in a range of plant developmental processes and in response to biotic and abiotic stresses. In the present study, a genome-wide comprehensive analysis of the *NAC* transcription factor gene family in quinoa was performed. In total, we identified 107 quinoa *NAC* transcription factor genes, distributed equally between sub-genomes A and B. They are phylogenetically clustered into two major groups and 18 subgroups. Almost 75% of the identified *CqNAC* genes are duplicated from two to seven times and the remaining 25% of the *CqNAC* genes are found as a single copy. Transcriptional responses of the identified quinoa *NAC* TF genes in response to various abiotic stresses were analyzed. Transcriptomic data revealed 28 stress responsive *CqNAC* genes where their expression significantly changed in response to one or more abiotic stress including salt, drought, heat and phosphate starvation. Among these stress responsive *NACs*, some *NACs* were previously known as stress responsive in other species indicating their conserved function in response to abiotic stress across plant species. Six genes were differentially expressed specifically in response to phosphate starvation but not to other stresses these genes may play a role in controlling plant responses to phosphate deficiency. Although additional phenotyping experiments with altered expression of these genes would help to precisely identify the biological function of these candidate genes, these results provided insights into quinoa *NACs* that could be used in future for genetic engineering or molecular breeding.

Session 6: RELATED TALKS

Poster presentation F2

First insights into the Oxidative Phosphorylation (OXPHOS) system of the halophyte *Cakile maritima* (Brassicaceae)

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Mitochondria play a central role in the energy metabolism of plants. At the same time, they provide energy for plant stress responses. We here present a first molecular characterization of the mitochondrial Oxidative Phosphorylation (OXPHOS) system of the halophile (salt tolerant) plant *Cakile maritima*. Mitochondria were purified from suspension cultures of *C. maritima* and for comparison of *Arabidopsis thaliana*, a closely related glycophyte (salt sensitive). Mitochondria were treated with digitonin and solubilized protein complexes were analyzed by two-dimensional (2D) Blue native / SDS polyacrylamide gel electrophoresis. The OXPHOS systems of the two compared plants exhibit some distinct differences. *C. maritima* mitochondria include a very abundant respiratory supercomplex composed of monomeric complex I and dimeric complex III. At the same time the complexes II and IV are of reduced abundance. The stability of the OXPHOS complexes was investigated by combined salt and temperature treatments. ATP synthase (complex V) is of increase stability in *C. maritima*. Also, the $I+III_2$supercomplex is present at high abundance during stress treatments. As a whole, our data provide insights into the contribution of mitochondria to the adaptive strategy of *C. maritima*, a plant native of saline and hot environments.

Session 6: RELATED TALKS

Poster presentation F3

Beneficial bacteria isolated from pioneer desert plants induce salt stress tolerance in Arabidopsis and increase the yield of Barley crops

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Deserts, such as those found in Saudi Arabia, are one of the most hostile places for plant growth. However, desert plants can impact their surrounding microbial community and select beneficial microbes that promote their growth under these extreme conditions. Here, we examined the bacterial communities in the soil, rhizosphere, and endosphere of four native desert plants collected from the Jizan region of Saudi Arabia. Interestingly, the endosphere compartment was less species-rich and diverse than other compartments. Culture-dependent isolation of the endosphere compartments yielded 116 bacterial isolates, of which many possessed plant growth promotion (PGP) traits, e.g., nutrient acquisition, abiotic stresses tolerance and promotion of salt stress tolerance on Arabidopsis plants. Preliminary screen qualified eleven bacterial isolates for further investigation on genome-sequence analysis of bacteria and impact on plant biomass, root system architecture, ion content distribution (Na^+, K^+), transcriptional changes (qPCR, dual RNA-seq), and field trials on important crops, e.g. barley. Our results revealed that despite their phylogenetic diversity and PGP traits some bacteria share commonalities in salinity stress tolerance promotion. Moreover, the changes in sodium and potassium levels in the shoot are a crucial mechanism by which PGPB induce salt stress tolerance in Arabidopsis. Under the desert farming conditions, inoculation of isolate JZ38 exhibited the ability to increase barley yield using saline irrigation, validating the promising potential of using desert microbes as a sustainable solution for alleviating the negative effects of abiotic stresses, such as salinity, on agricultural productivity.

Session 6: RELATED TALKS

Poster presentation F4

Quinoa Rehamna project: Scaling up quinoa value chain to improve food and nutritional security in poor rural communities of Morocco

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Quinoa is a revenue-generating crop that has the potential to improve the livelihoods of poor smallholder farmers in critical conditions. As such, it is well suited for addressing income challenges faced by smallholder farmers of marginal areas of Rehamna in Morocco. Quinoa was introduced in Morocco in 2008, however its production has been constrained by lack of access to well-adapted and high-yielding cultivars, inappropriate crop management practices, weak value chains and limited market demand. The proposed project is implemented in Rehamna Province, where a quinoa value chain already exists but is constrained by various factors. The project will analyze the existing quinoa value chain, develop a pro-poor business model and scale it up. It will introduce and disseminate quinoa cultivars with high tolerance to abiotic stresses, high productivity and yield stability, as well as promote appropriate crop production/management practices among smallholder quinoa growers. It will propose solutions to increase the demand for quinoa-based. This project is implemented in two phases. During the first phase, a business model will be developed and tested during a period of one year and half. Based on the outcomes of the pilot phase, a scaling up strategy is under development and will be implemented during the second phase, spanning one year and a half. A survey of 12 communes of Rehamna was undertaken to assess the existing quinoa value chain, and to identify gaps and weaknesses at all stages. The results of conducted survey indicated great interest of local farmers, women's coop and local institution in developing the Quinoa value. ICBA in collaboration with UM6P introduced new productive lines within nine farmers in Bouchane commune to be compared to the local line and tested good practices to increase Quinoa productivity. Obtained results show that ICBA accessions demonstrated very good performance compared to locally cultivated lines.

Key words: Seed processing, Quinoa based-food, Irrigation, Amendment, Yield, Couscous

Session 7

NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Session 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Oral presentation

The senescence associated barley cysteine protease HvPAP14 is targeted to chloroplasts and contributes to the degradation of the photosynthetic apparatus

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Chloroplasts contain the highest proportion of the total leaf protein content. The degradation of chloroplast proteins therefore is of pivotal importance for remobilization of nitrogen during leaf senescence. The detection of senescence associated genes encoding cysteine proteases is in accordance with the idea that bulk degradation of chloroplast proteins during senescence occurs in vacuoles. In barley (*Hordeum vulgare* L.) HvPAP14 belonging to the C1A family of cysteine proteases was found to have highest gene expression in senescent flag leaves (Hollmann et al., 2014). The enzyme was identified in senescing barley leaves by affinity enrichment using the mechanism-based probe DCG-04 targeting cysteine proteases. The HvPAP14:RFP fusion protein was detected in the endoplasmic reticulum and in vesicular bodies. Immunological studies showed, however, that HvPAP14 is mainly located in chloroplasts where it was found in tight association with thylakoid membranes. The recombinant enzyme is activated by low pH being in accordance with the detection of HvPAP14 in the thylakoid lumen. Overexpression of HvPAP14 in barley revealed that the protease can cleave thylakoid LHC proteins and lumenal PSBO as well as the large subunit of Rubisco. In barley plants overexpressing a HvPAP14:RFP construct, fluorescence was shown to be associated with vesicular structures which seem to emanate from chloroplasts. Immunogold labelling identified vesicles that besides HvPAP14 contain also PSBO as shown for the CV-containing vesicles (CCVs) proposed to transport proteins for degradation to the vacuole through a pathway that is independent of autophagy and senescence associated vacuoles (Wang and Blumwald 2014).

Key words: Chloroplasts, Protein degradation, Barley, Cysteine proteases, Microscopy

Session 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Oral presentation

Nucleotide catabolism recycles nucleobase nitrogen

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Plants recycle nutrients, for example nitrogen, during senescence. Most nitrogen is stored in proteins, but some is present the nucleobases of nucleic acids and nucleotides. Our research is focused on the elucidation of the enzymatic players and biochemical pathways of nucleotide catabolism in plants. Recently, we analyzed the network of purine nucleotide catabolism using targeted metabolite profiling in a multitude of mutants and assessing the biological role of a nucleoside hydrolase with so far unknown function. We also identified the first nucleotide phosphatase involved in nucleotide catabolism in vivo, which possibly acts as a gatekeeper to give access to purine nucleotide catabolism for nutrient recycling and to supply intermediates of purine nucleotide degradation required under stress conditions. Based on our results, a profoundly revised model of purine nucleotide catabolism in plants is presented.

Key words: Nucleotide catabolsim, Enzyme discovery, Biochemical pathway discovery

Session 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Oral presentation

Evolutionary aspects of chlorophyll breakdown

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The degradation of chlorophyll is the most obvious visual symptom of leaf senescence. Chlorophyll is broken down in a pathway, termed the PAO/phyllobilin pathway, that involves numerous steps and intermediates. It can be divided in two parts: the first leading from chlorophyll to a primary fluorescent chlorophyll catabolite, which in subsequent steps is modified at several peripheral side positions to form a mixture of degradation end products, termed phyllobilins, that accumulate in the vacuoles of senescent leaves. While the first part of the pathway seems to be conserved across species, phyllobilin modification reactions are species-specific as deduced from the analysis of phyllobilins from nearly 30 plant species.
The aim of this work is to elucidate the evolutionary origin of the pathway and to investigate how much the PAO/phyllobilin pathway is conserved within non-angiosperm organisms, especially oxygenic photosynthetic species. There is a decreasing sequence identity to the *Arabidopsis thaliana* proteins of the PAO/phyllobilin pathway in early evolved plant species - compared to more recently originated ones. In addition, in some groups, such as the Charophytes and Cyanobacteria, it is unknown whether chlorophyll breakdown takes place at all or which types of phyllobilin modifications occur.
Using some key species along the green lineage, we are able to show that phyllobilins already occur in green algae, specifically in *Auxenochlorella protothecoides*, and in the liverwort *Marchantia polymorpha*. Based on RNAseq experiments of green and senescent A. protothecoides cultures we identified a potential PAO (ApPAO), which is up-regulated during degreening. Using respective A. thaliana mutants, we are now in the process to test functionality of the potential ApPAO and of other chlorophyll catabolic enzyme candidates from *A. protothecoides*, *M. polymorpha* and *Klebsormidium flaccidum*, the proposed direct ancestor of land plants.

Key words: Chlorophyll breakdown, Phyllobilins, green lineage, *Auxenochlorella protothecoides*, *Klebsormidium flaccidum*

Session 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Oral presentation

Arabidopsis Ubiquitin-specific Proteases UBP12 and UBP13 shape ORE1 levels during leaf Senescence induced by nitrogen deficiency

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Nitrogen deficiency in plants triggers leaf senescence which is regulated by the transcription factor ORE1. We have recently reported that the Arabidopsis NLA (Nitrogen Limitation Adaptation) E3 ligase down-regulates ORE1 to attenuate leaf senescence. Here, we show that UBP12/UBP13 (Ubiquitin-specific protease 12/13) antagonize the action of NLA to maintain ORE1 homeostasis.

Like ore1, ubp12-2w/13-3 shows delayed leaf senescence compared to WT under normal aging or nitrogen deficient (–N) conditions. UBP12 and UBP13 bind to ORE1 in vitro and in vivo with specificity and deubiquitinate polyubiquitinated-ORE1. We analyzed in various genotypes total chlorophyll content and expression levels of senescence-related genes under –N conditions. Plants overexpressing UBP12/UBP13 display accelerated leaf senescence which is reversed by the ore1 mutation. By contrast, the senescence phenotype of ORE1-overexpressed plant is exacerbated by UBP12/UBP13 overexpression. Expression of senescence-related genes is parallel the senescence phenotype. ORE1 protein levels are increased by MG132 suggesting its instability is due to ubiquitin-mediated degradation. At similar ORE1 transcript levels, ORE1 protein levels can be elevated by UBP12/UBP13 overexpression but decreased in ubp12-2w/13-3. Taken together, our results show that UBP12/UBP13 deubiquitinate ORE1 to maintain its levels and promote the activity of ORE1 as positive regulators for leaf senescence in –N response

Key words: Leaf senescence, Nitrogen deficiency, UBP12, UBP13, ORE1, Deubiquitination

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Oral presentation

Natural variations of the Stay Green gene promoter control lifespan and yield in rice cultivars

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Two major subspecies of rice, indica and japonica, show drastically different lifespans. Here, we found that, by quantitative trait locus mapping, variations of the promoter but not the coding region of the Stay Green (*OsSGR*) gene on chromosome 9 is responsible for shorter lifespans of indica rice through earlier and higher induction of *OsSGR* encoding chlorophyll-degrading Mg++-dechelatase. The indica-type promoters are present in a progenitor subspecies *O. nivara* and thus was acquired early during the evolution of rice subspecies for rapid cycling traits. Japonica *OsSGR* alleles introgressed into high-yield indica-type cultivars lengthened their lifespan and further increased grain yield (up to 10%) in Korean rice fields. Thus, the *OsSGR* promoter variations and the related lifespan variations are a key ecological and agronomic trait in rice evolution and breeding.

Key words: Rice, Stay Green, Lifespan, Promoter, Productivity

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Poster presentation G1

Responses to darkness senescence and low nitrate availability of nitrogen use efficiency in *Arabidopsis thaliana*

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Nitrogen (N) is an essential nutrient that plants require for the synthesis of amino acids, proteins, and many other important metabolites. Plant metabolism and growth are consequently dependent of the amount of N that is assimilated and distributed from source leaves to developing sinks, like fruits and seeds. Environmental stress, such as low nitrate availability and darkness senescence, deeply influence seed yield and seed composition in plants. Carbon (C) and N metabolisms are highly interrelated but C and N partitioning are complex traits with low or intermediate heritability and strong genotype x environment interactions. Our research aims to compare the plant response to low nitrate and to early leaf senescence. By using a collection of genotypes showing a range of C:N ratio in seeds, we investigated the impact of different post flowering conditions (control, low nitrate availability and induced senescence) onto seed yield, N allocation in organs, NUE, N remobilization efficiency and N uptake. We developed a statistical method to explore the C:N stoichiometry in seeds to analyze how the post-flowering stress could change the seed filling. Altogether, the data indicate that the response to environmental stress generates different bottlenecks on nitrogen fluxes during the seed filling.

Key words: Nitrogen remobilization efficiency, Darkness senescence, Seed filling, Harvest index

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Poster presentation G2

Is nitrogen relocated during fine root senescence in *Populus trichocarpa*

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Senescence is a precisely controlled process that follows in a strictly defined order and may influence a wide range of the ecological processes. It is characterized by the shift from nutrient assimilation to nutrient remobilization. Mechanisms related to nitrogen remobilization are quite well documented during leaf senescence. It was noted that glutamine synthetase (GS) is a crucial enzyme which enables nitrogen relocation from senescent tissues to other parts of plants. However, there is a gap in our knowledge concerning both senescence as well as remobilization in another ephemeral organs - fine roots. Considering that the biomass of fine roots is equal to or greater than leaf biomass, relocation of nitrogen from senescent fine roots seems to be an important issue that may influence biological processes in the forest ecosystems.

The aim of our study was to verify the hypothesis that nitrogen is relocated during fine root senescence and the mechanisms of remobilization are similar to those described in leaves. We performed quantitative analyses of nitrogen content and expression of genes encoding GS, localization of those transcripts in the examined organs, as well as we detected GS protein. Our studies revealed that the nitrogen content decreased during senescence of fine roots. Interestingly, one of the duplicate genes encoding GS was upregulated and GS content was elevated in senescent fine roots. These results suggest that nitrogen might be actively retranslocated from senescent tissues of fine roots. This is the first molecular study that provides the evidence of nitrogen remobilization during fine root senescence. Moreover, we confirmed that the pathway of nitrogen relocation is similar as in leaves, what is another common feature of those organ senescence.

Key words: Nitrogen remobilization, Fine roots, Absorptive roots, Senescence, Glutamine synthetase

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Poster presentation G3

Interaction of leaf aging to iron nutrition in the iron homeostasis of chloroplasts

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In chloroplasts iron (Fe) is required for the development and function of the photosynthetic apparatus. The reduction-based Fe uptake of chloroplasts is thought to be operated by PIC1-NiCo complex, whereas the action of FRO7 ferric chelate reductase is also essential. MAR1 were also associated to Fe uptake and homeostasis of chloroplasts previously. Although some components of the uptake machinery is known already, the regulation of the system has not characterized yet. *Brassica napus* of deficient, optimal and superoptimal Fe nutrition status were subjected to quantification studies of FRO7, MAR1, PIC1 and NiCo during and after the leaves reached their full maturity. Fe and chlorophyll contents together with the function of the photosystem II (PSII) were used to model the physiological status of chloroplasts. Fe deficiency not only retained the Fe accumulation in chloroplasts, but induced an early increase in the inactivation of PSII. In parallel, PIC1 and FRO7 showed a repression by reaching the full maturity, whereas NiCo and MAR1 became upregulated. In contrast, superoptimal Fe supply lead to the delays of these signs of senescence: PSII activity and Fe content remained stable even in older leaves. In conclusion, NiCo and MAR1 are suggested not participating in Fe uptake. A small but tendentious decrease in the Fe content is coupled to the aging of non Fe-overloaded plants' leaves, whereas a delayed senescence is coupled to a stable Fe homeostasis under superoptimal Fe nutrition. Nevertheless, the priority of internal senescence signals and the organism-level need for Fe in the alterations of chloroplast Fe homeostasis has not been resolved yet.

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Key words: Chloroplast, FRO7, MAR1, NiCo, PIC1

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Poster presentation G4

Constancy in nuclei DNA content in senescing leaves at the time of abscission in two deciduous trees

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Leaves of two deciduous tree species were sampled at green and at senescent stage after abscission. Flow cytometry showed that the size of the nuclei of leaf cells did not change after the senescence process and that a large number of nuclei were recovered after abscission as well as on mature green leaves. The preservation of genomic DNA after abscission indicates the absence of resorption of the nutrients contained in DNA molecules and, most likely, also in histones and other nuclear proteins as well as in phospholipids of the nuclear membrane. This consideration has implications for the interpretation of the role that PCD mechanisms associated to the late steps of leaf senescence have in the remobilization of nutrients and of its time overlap with leaf abscission.

Key words: Nuclei, Flow cytometry, Nutrient resorption

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Poster presentation G5

Transcriptomic analysis of leaf senescence and nitrogen deficiency in *Brassica napus*: a focus on proteolysis

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Improving nitrogen use efficiency (NUE) is of major interest as low nitrogen-inputs cultural practices have to be developed to face economic and environmental issues. Reduction of nitrogen (N) fertilizers without dramatically affecting grain yields may be achieved by improvement of N uptake, translocation, assimilation or remobilization of crops. Rapeseed is characterized by a low NUE mainly resulting from inefficient N remobilization during leaf aging. Thus, studies on nutrient recycling processes during leaf senescence should provide interesting perspectives for NUE improvement. For this purpose, a transcriptomic analysis of leaf development and senescence-associated adjustments under N deficiency was achieved.
In the winter oilseed rape cultivar Aviso grown under optimal N supply or mild N deficiency, the transcriptome changes associated with the leaf development were investigated at the silique filling stage. RNA sequences from senescent and mature fully developed leaves obtained in both N regimes were compared. Around 4000 differentially expressed genes could be identified, representing potential targets for the leaf nutrient remobilization improvement in the context of low N inputs practices. Gene ontology analysis did highlight important regulations occurring at N metabolism, transmembrane transport processes and proteolysis in senescing leaves, reflecting the N recycling processes engaged. Here, a focus on the proteases involved in *B. napus* leaf senescence and N deficiency is proposed. A detailed comparison of our results with those obtained through similar approaches performed on rapeseed (Safavi-Rizi et al., 2018) and *Arabidopsis thaliana* (Breeze et al., 2011) gives an overview of the major protease families involved in N recycling and remobilization during leaf senescence in *B. napus* and offers new breeding targets for yield improvement.

Key words: Nitrogen use efficiency, Nitrogen remobilization, Source-sink relationships, Protease

SESSION 7: NUTRIENT RECYCLING AND PLANT PRODUCTIVITY

Poster presentation G6

Diversity among stay-green and senescent sorghum accessions revealed by SNP's and proteome analysis

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Drought stress is one of the major constraints to crop production and food security worldwide. Exploration of plant variation in response to drought stress will provide valuable information to develop drought tolerant plants. In this study, the genetic diversity of 8 sorghum accessions of known senescence phenotype was investigated using Single Nucleotide Polymorphism's distributed across the sorghum genome. The genetic distance of accessions ranged between 0.22 and 0.53. The most diverse accessions were the senescent high yielded cultivar Tabat and the stay-green accession HSD8266. Proteomic analysis of the stay green accessions under polyethylene glycol stress revealed differentially expressed proteins related to key biological processes (photosynthesis, various metabolic pathways and other molecular regulatory processes). The tested accessions showed genetic variability for the traits considered. These genetic sources of stay-green could provide a valuable resource for improving this trait in sorghum breeding programs.

Session 8

POSTHARVEST PHYSIOLOGY AND SENESCENCE

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Oral presentation

New insights into the molecular control of postharvest senescence through study of Arabidopsis inflorescences

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Energy deprivation, wounding and disruption of water and nutrient supply are stresses that have the potential to cause the early deterioration of detached chlorophyllous tissues held in the dark. To identify the key stress signalling biology responsible for the precocious senescence of harvested tissues we developed a simple assessment system based on the degreening of detached dark-held immature Arabidopsis inflorescences. Using this system in combination with transcriptome profiling and forward and reverse genetics approaches we found that carbon deprivation-mediated metabolic reprogramming is a large part of the postharvest response. There is also a complex additive interplay between the major hormonal pathways and the progression of senescence. As found for other biological transitions, our most recent results suggest that postharvest deterioration may require a change in tissue competency before it can be initiated, which is governed by particular transcription factors. Our results, together with those of other researchers, also suggest that the same key regulators that control dark-mediated senescence in a detached system drive other major developmental programmes, including age-related leaf senescence, fruit ripening and stress response. Thus it will be argued that study of 'artificial' detached systems can be the most efficient route to understanding the key biology behind these diverse fundamentally important programmes.

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Oral presentation

Metabolic and transcriptomic shifts during tobacco leaf post-harvest senescence

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Tobacco is harvested after plant flowering, when the leaf senescence is more or less visible depending on cultivars. Before the drying phase, current practices allow the senescence to proceed until the detached leaf becomes yellow. This yellowing phase is known to be critical for the final properties of the leaf. This phase was analysed for chemical and expression changes in leaves of cultivars belonging to the three main tobacco types, Virginia, Burley, and Oriental, grown according to their respective practices. In line with important metabolic changes, we observed a significant transition in the genetic program, including chlorophyll degradation, amino acids, and reducing sugar production within hours following the field harvest. We found that in Burley and Oriental tobacco varieties, certain amino acids, such as asparagine, are rapidly synthesized upon detachment, whereas the Virginia tobacco variety mainly produces reducing sugars. Genes differentially expressed after two days of leaf senescence were identified. Downregulated genes mostly belong to metabolic pathways, such as oxidation reduction, carbohydrate and lipid metabolism, and photosynthesis (e.g., RuBisCO subunits). On the other hand, upregulated genes are related to redox reactions, transcription regulation, proteolysis, hydrolase activity, and other catabolic processes. This finding is consistent with previous studies depicting leaf senescence processes. Go-terms analysis performed after 48 hours allowed identification of key transcripts involved in sugar and amino acid metabolism pathways, which may explain some major observed biochemical changes linked to the redistribution of carbon and nitrogen resources during tobacco leaf senescence.

Key words: leaf properties, manipulating senescence, biochemistry, gene expression, knowledge acquisition

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Oral presentation

Discovering the genes that are involved in postharvest senescence in broccoli (*Brassica oleracea*)

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The plant hormone abscisic acid (ABA) plays an important role in many processes related to survival in stressful environments. ABA can inhibit formation and growth of leaves and is involved in stress induced senescence. We have recently identified a transcription factor ABIG1 that is necessary for stress-induced senescence in Arabidopsis (Liu et al, eLife, 2016). We are exploring the regulatory networks through which ABIG1 act to control growth in Arabidopsis as well as in the cultivated Brassica plants species using systems biology, quantitative genetic, and comparative genomics approaches. The cultivated Brassica species, which are the group of crops most closely related to Arabidopsis. Previous comparative research between *Brassica oleracea* and *Arabidopsis thaliana* genome identified numerous one-to-one segmental relationships and genome duplications. This is particularly interesting because little is known about the molecular mechanism of postharvest senescence in Brassica species including broccoli, cabbage, cauliflower. To carefully characterize the effects of environmental stresses on postharvest broccoli, we are working on identifying candidate transcripts, related proteins and metabolic compounds that accurately reflect the physiological age or freshness of broccoli and the other Brassica species during postharvest storage through transcriptomics, proteomics and metabolomics approaches. Identifying those 'fresh-indicators' that has the potential to mediate senescence and to generate germplasm for breeding new varieties with stress tolerance and postharvest color retention in vegetables.

Key words: Postharvest genomics, Stress responses, Gene networks, Broccoli, Senescence

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Oral presentation

Retardation of cut flower senescence by regulation of anthocyanin pigmentation: Role of light, sugar, and developmental stage

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Cut flowers with anthocyanin pigmentation, harvested with inflorescences bearing florets at various developmental stages, complete their development in the vase under low light intensity (14-20 μmols), but with a significantly reduced color than that obtained under cultivation light intensities (700-1300 μmols). Addition of sugar to the vase solution partially improved petal pigmentation, but color was still faded. Thus, flower opening and anthocyanin pigmentation in the vase, which determine the ornamental value, are restricted by two main factors, low light and carbohydrates deficiency, as full anthocyanin biosynthesis requires high light. In order to study this problem, we investigated four cut flowers of anthocyanin-pigmented species, which suffer from the reduced color problem in vase life: Lisianthus (*Eustoma grandiflorum*), *Delphinium elatum*, *Phlox paniculata* and snapdragon (*Antirrhinum majus*). The anthocyanin levels of these cut flowers increased with increasing light intensity (0 to 500 μmols), but they responded differently to sugar supply in the vase solution. Thus, the enhancing effect of sugar on anthocyanin levels of Delphinium and Phlox was pronounced in darkness, while under low light, sugar either had no additional enhancing effect on the light-induced anthocyanin level (Delphinium), or it had a synergistic effect (Phlox and Lisianthus). The reduced anthocyanin pigmentation of these flowers in response to low light resulted from the down-regulated expression of early and late anthocyanin biosynthesis genes, depending on the flower developmental stage upon transfer to low light. This suggests that light intensity regulates one or more master transcription factors (TF) common for all these genes. Indeed, the results obtained with snapdragon and Phlox flowers show that the MYB TF, which co-regulates anthocyanin biosynthesis, responded to low light and/or sucrose supply in vase solution. Based on these findings, biotechnological tools can be developed to control anthocyanin biosynthesis, which may result in fully pigmented flowers under indoors low light intensity.

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Oral presentation

Short-term stress affects profiles of volatile organic compounds and gene expression in rocket salad during postharvest senescence

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Once harvested, leaves undergo a process of senescence which is distinct from developmental senescence, but has some overlapping features. These include changes in gene expression, a loss of photosynthetic capacity and metabolite changes. Of particular interest in fresh produce are changes in nutrient content, and aroma, which is dependent on the profile of volatile organic compounds (VOCs) produced. Leafy vegetables are subjected to multiple stresses during and shortly after harvest. These include mechanical damage, storage or transport under different temperature regimes and low light conditions. These are thought to impact on later shelf-life performance by altering the progression of postharvest senescence. Short term stresses in the first 24 h after harvest were simulated in wild rocket (*Diplotaxis tenuifolia*). These included dark at ambient temperature, dark and wounded at ambient temperature and 4°C in the dark. Effects of stresses were monitored immediately afterwards and after one week of storage at 8°C, a realistic temperature for the industry. Gene expression changes in two NAC transcription factors (orthologues of *ANAC059* and *ANAC019*), and in a gene involved in isothiocyanate production (thiol methyltransferase, TMT) were evident immediately after the stress treatments and some persisted also following week of storage. Vitamin C loss and microbial growth on the leaves were also affected by the stress treatments. VOC profiles were differentially affected by the stress treatments and the storage period. Overall, evidence is provided that short term stress postharvest, affects multiple aspects of rocket leaf postharvest senescence even after a period of cold storage. However, different stress combinations elicit different responses.

Key words: Rocket salad, Volatile organic compounds, Stress memory, Nutritional content, Gene expression

SESSION 8: POSTHARVEST PHYSIOLOGY AND SENESCENCE

Poster presentation H1

A transcriptome analysis of peduncle necking in cut *Rosa hybrida* cultivar 'H30'

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Bending of the peduncle or 'necking' is a primary cause of reduced vase life for roses, and therefore a major issue for the cut flower industry. Necking is thought to be symptom of water stress, caused by a blockage of the xylem, either due to an air embolism or a microbial accumulation at the stem end. The incidence of necking however varies widely between cultivars, with certain cultivars being more susceptible than others. A transcriptome analysis of the susceptible *Rosa hybrida* cultivar 'H30' has therefore been carried out to investigate the potential molecular mechanisms involved. Peduncle samples of three stages of necking (straight, <90° and >90°) were sequenced using next generation sequencing to produce over 100 million reads per stage. Following alignment to the *Rosa Chinensis* 'Old Blush' genome, over 3,500 differentially expressed genes (p adjust <0.05) have been identified and explored; providing a great new resource for the understanding of this postharvest issue.

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