Content

POG 2019

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Helmholtz Zentrum München
German Research Center for Environmental Health
Ingolstädter Landstrasse 1
85764 Neuherberg, Germany
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Conference Venue

Münchner Künstlerhaus
Lenbachplatz 8 | 80333 Munich, Germany
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The Conference Venue offers free WiFi access.
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Conference Language

The official conference language is English.

Conference Dinner on Thursday, July 11

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Conference Program

Wednesday, July 10, 2019

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	Plant Oxygen Group Meeting	

Thursday, July 11, 2019

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Friday, July 12, 2019

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Abstracts

Talks

T 1 | Wednesday, July 10, 2019, 9:30 - Session I

FERROPTOSIS, A METABOLIC DEATH PATHWAY

M. Conrad

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Ferroptosis is a regulated form of necrotic cell death highly relevant for a number of (neuro)degenerative diseases and cancer. Ferroptosis-like cell death has also been described in higher plants, indicating that this form of cell death is shared among complex organisms. Hallmark of ferroptosis is iron-dependent lipid peroxidation causing caspase- and RIPK-independent cell death. Due to its unique role in quenching lipid peroxidation in membranes, the selenoenzyme glutathione peroxidase 4 (GPX4) is now regarded as the key regulator of this form of cell death. Yet, long before the term ferroptosis was coined we could show that neuron-specific GPX4 ablation causes ataxia and neurodegeneration in hippocampus of newborn mice, which evidently occurred in a non-apoptotic form of cell death. Meanwhile, genome-wide CRISPR/Cas screens have introduced acyl-CoA synthetase long chain family member 4 (ACSL4) as an additional member of the ferroptotic process. The role of ACSL4 in the ferroptotic process relies on its activity to activate preferably long chain, polyunsaturated fatty acids, which, when incorporated into lipid bilayers, may undergo peroxidation leading to the generation of proximate signals of ferroptotic cell death. Current studies are geared towards a better understanding of the relevance of iron-dependent lipid peroxidation and ferroptosis in human disease, the preclinical development of ferroptosis modulators and the discovery of novel players in the ferroptosis process.

T 2 | Wednesday, July 10, 2019, 10:00 - Session I

CONCEPTS AND DIRECTIONS IN REDOX SIGNALLING IN PLANTS

C. H. Foyer

School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, Edgbaston, B15 2TT, UK

Our understanding of the functions of reactive oxygen species (ROS) has been entirely revised over recent decades. Initially confined to oxidative stress and associated cellular damage ROS are now recognised as signals released from the plasma membrane and organelles to orchestrate plant growth and stress tolerance. Changes in ROS production alter the reduction/oxidation (redox) status of plant cells, exerting a strong influence on metabolism and the control of gene expression. ROS act as a signals through the redox processing of other molecules particularly proteins. Reactively little is known about the network of proteins that are undergo redox-mediated post translational modifications, highlighting the need for improved redox proteomics approaches. While ROS can only trigger oxidative processing of proteins and other molecules, redox-signalling also encompasses the reductive processing of molecules. In this talk, cellular redox homeostasis will be considered as an ‘‘integrator’’ of information from metabolism and the environment in the control of plant stress responses. Low molecular antioxidants (e.g., ascorbate, glutathione) serve not only to limit the lifetime of the ROS signals but also to participate in an extensive range of other redox signalling and regulatory functions. This talk will focus on the role of antioxidants and redox-regulated proteins in the orchestration of plant stress responses.

T 3 | Wednesday, July 10, 2019, 11:20 - Session I

THE EVOLUTION OF NITRIC OXIDE SIGNALLING DIVERGES BETWEEN THE ANIMAL AND THE GREEN LINEAGES

J. Astier [1], A. Mounier [1], J. Santolini [2], S. Jeandroz [1] and D. Wendehenne [1]

Agroécologie, AgroSup Dijon, CNRS, INRA, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France [1]; Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ Paris-Sud, Université Paris-Saclay, F-91198, Gif-sur-Yvette cedex, France [2].

Nitric oxide (NO) is a ubiquitous signalling molecule with widespread distribution in prokaryotes and eukaryotes where it is involved in countless physiological processes. While the mechanisms governing NO synthesis and signalling are well established in animals, the situation is less clear in the green lineage. Recent investigations have shown that NO synthase (NOS), the major enzymatic source for NO in animals, is absent in land plants but present in a limited number of algae. First detailed analysis highlighted that these new NOSs are functional but display specific structural features and probably original catalytic activities. Completing this picture, analyses were undertaken in order to investigate whether major components of the prototypic NO/cyclic GMP signalling cascades mediating many physiological effects of NO in animals were also present in plants. Only few homologues of soluble guanylate cyclases, cGMP-dependent protein kinases, cyclic nucleotide-gated channels and cGMP-regulated phosphodiesterases, were identified in some algal species and their presence did not correlate with that of NOSs. In contrast, GSNO reductase, a critical regulator of S-nitrosothiols, was recurrently found. Overall, these findings highlight that plants do not mediate NO signalling through the classical NO/cGMP-signalling module and support the concept that S-nitrosation is a ubiquitous NO-dependent signalling mechanism.

T 6 | Wednesday, July 10, 2019, 14:30 - Session II

YES TO NO? BIOSENSORS

E. Eroglu[1], H. Bischof[1], S. Hallstroem[2], M. Waldeck-Weiermair[1], W.F. Graier[1], and R. Malli[1]

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[2]Physiological Chemistry, Otto Loewi Research Center, Medical University of Graz, Neue Stiftingtalstraße 6/2, 8010 Graz, Austria

Nitric Oxide (NO) is a ubiquitous signaling molecule, which modulates diverse biological functions in bacteria, animals, and plants. Depending on the duration, localization, and concentration, NO can also contribute to disease initiation, progression, and severity in cells, tissues, and organs. Because of its short-lived nature and rapid reactivity, NO is difficult to measure directly with high accuracy and precision. Thus, there are still considerable gaps in our understanding of subcellular NO signals in health and diseases. We recently advanced a novel class of fluorescent protein (FP) –based biosensors - termed geNOps - which permit real-time imaging of NO signals on the level of individual cells and subcellular locals. The geNOp sensors are simply engineered chimera, which consists of a single FP that is fused to a bacteria derived non-heme iron(II) binding domain (GAF domain) which in turn is capable of interacting with NO reversibly. NO binding to the GAF domain instantly affects the spectral properties of the FP, leading to a decrease in brightness in a reversible manner. Thanks to their distinct spectral properties, geNOps also allow real-time recordings of local NO signals simultaneously along with other fluorescent probes. The geNOps emerged as valuable tools that have the potency to gain our understanding of the complex biochemistry of NO in cells of different species.

T 7 | Wednesday, July 10, 2019, 15:00 - Session II

THE JANUS FACE OF PLANT HYPOXIA

B. Giuntoli[1,2], D. Weits[2] & F. Licausi [1,2]

Department of Biology, University of Pisa, Italy; Plantlab, Institute of Life Science, Scuola Superiore Sant'Anna, Pisa, Italy

Availability of molecular oxygen (O_2) is not uniform for all biological life forms on Earth, and actual oxygen abundance varies greatly across tissues of multicellular organisms such as plants and animals. This entails multiple sensing mechanisms, based on direct or indirect oxygen perception, to adapt growth and development to oxygen levels. Remarkably, the evolution of animals and plants converged towards the exploitation of selective proteolysis to control transcriptional regulators in an oxygen dependent manner. The similarity between the two kingdoms of life is not limited to this: our recent results also indicate that plant meristems require the maintenance of hypoxic niches for their function, similarly to what observed in certain types of mammal stem cells. Our observations led us to speculate that, in plants, a cysteine-branch of the N-degron pathway subdued different proteins to oxygen-dependent control, possibly to attune metabolism and development to environmental cues. As a proof of concept, we also succeeded in imposing hypoxia-dependent regulation to chimeric proteins in buddying yeast by introducing the enzyme involved in oxygen dependent channelling towards proteasomal degradation. The consequences of this convergence in the context of evolution of multicellularity and the new avenues to exploit this regulation will be presented.

CHLOROPLASTS REQUIRE GLUTATHIONE REDUCTASE TO BALANCE REACTIVE OXYGEN SPECIES AND MAINTAIN EFFICIENT PHOTOSYNTHESIS

S. J. Müller-Schüssele[1], R. Wang[2], D. D. Gütle[3], J. Romer[4], M. Rodriguez-Franco[5], M. Scholz[2], V. M. Lüth[3], S. Kopriva[6], P. Dörmann[4], M. Schwarzländer[2], R. Reski[3,7], M. Hippler[2], A. J. Meyer[1]

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Thiol-based redox-regulation is vital to coordinate chloroplast functions depending on illumination. Yet, how the redox-cascades of the thioredoxin and glutathione redox machineries integrate metabolic regulation and reactive oxygen species (ROS) detoxification remains largely unresolved. We investigate if maintaining a highly reducing stromal glutathione redox potential (E_{GSH}) via glutathione reductase (GR) is necessary for functional photosynthesis and plant growth.

Since absence of the plastid/mitochondrial GR is embryo-lethal in *Arabidopsis thaliana*, we used the model moss *Physcomitrella patens* to create knock-out lines. We dissect the role of GR in chloroplasts by *in vivo* monitoring stromal E_{GSH} dynamics, and reveal changes in protein abundances by metabolic labelling.

Whereas stromal E_{GSH} is highly reducing in wildtype and clearly responsive to light, the absence of GR leads to a partial oxidation, which is not rescued by light. Photosynthetic performance and plant growth are decreased with increasing light intensities, while ascorbate and zeaxanthin levels are elevated. An adjustment of chloroplast proteostasis is pinpointed by the induction of plastid protein repair and degradation machineries.

Our results indicate that the plastid thioredoxin and glutathione redox systems operate largely independently. They reveal a critical role of GR in maintaining efficient photosynthesis.

S-NITROSOTHIOLS AS ARCHITECTS OF THE HISTONE PTM PATTERN AND DNA-METHYLATION IN *ARABIDOPSIS THALIANA*

E.-E. Rudolf[1], A. Ageeva-Kieferle[1], A. Mengel[1], P. Hüther[2], I. Forné[3], R. Hell[4], A. Imhof[3], M. Wirtz[4], C. Becker[2], J. Durner[1,5], C. Lindermayr[1]

Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, German Research Center for Environmental Health, Germany[1]; Gregor Mendel Institute[2]; Centre for Organismal Studies, Ruprecht-Karls-Universität Heidelberg, Germany[3]; Protein Analysis Unit, Ludwig-Maximilians-Universität München, Germany[4]; Chair of Biochemical Plant Pathology, Technische Universität München, 85354 Freising, Germany[5]

Nitric oxide (NO) is a reactive radical that regulates many physiological processes in plants. It acts by modulating target proteins through post-translational modifications such as S-nitrosylation, tyrosine nitration, or metal nitrosylation thereby altering their activity and/or function. A regulatory function of NO in epigenetic processes has already been reported. The major methyl donor for methylation of DNA and histones is S-adenosylmethionine (SAM) provided by the methylation cycle. In *Arabidopsis thaliana* S-adenosylmethionine synthetase (SAMS) and S-adenosylhomocysteine hydrolase (SAHH) were identified as targets for S-nitrosylation. *In vitro* studies demonstrated that SAMS isoform 1 and both isoforms of SAHH are inhibited by the physiological NO-donor S-nitrosoglutathione (GSNO). Interestingly, SAM increased in plants with enhanced endogenous S-nitrosothiol levels (*gsnor1-3*, loss of GSNOR function) and resulted in increased methylation index (MI = SAM/SAH). The MI is an important measure of the organismal methylation status and even small changes in the MI result in changes in transmethylation activity. Immunological detection of H3K9me2 and a nano liquid chromatography-tandem mass spectrometry profiling approach demonstrated independently a significant global increase in this modification. Since H3K9me2-modified regions are tightly correlated with methylated DNA regions, DNA methylation was analysed in wild type and *gsnor1-3* plants. Whole genome bisulfite sequencing revealed stronger DNA methylation in CG, CHG, and CHH context in *gsnor1-3* in comparison to wild type. Our data suggest that S-nitrosothiols are involved in regulation of the repressive chromatin mark H3K9me2 which is associated with the silencing of repeats and transposon elements.

T 8 | Wednesday, July 10, 2019, 15:30 - Session II

THE ROS WAVE: ITS REAL-TIME WHOLE-PLANT DETECTION, FUNCTION AND CHARACTERIZATION

R. Mittler, Y. Fichman, A. R. Devireddy and S. I. Zandalinas

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Reactive oxygen species (ROS) are key regulators of subcellular, cellular and systemic signals in plants and other organisms. We recently developed a robust and straightforward method for the whole-plant, real-time, *in vivo* detection of ROS, in plants grown in soil. Using this method we were able to determine some of the underlying mechanisms involved in initiating and driving the progression of the ROS wave, as well as study the involvement of the ROS wave in coordinating systemic stomatal responses. In addition to studying the ROS wave, the developed method could be used to study different genetic variants, conduct large-scale phenotyping studies and unravel additional routes of ROS signaling in plants and other organisms.

T 9 | Wednesday, July 10, 2019, 16:20 - Session II

MINING FOR PROTEIN S-SULFENYLATION IN *ARABIDOPSIS THALIANA* UNCOVERS NEW REDOX-SENSITIVE SITES

J. Huang[1,2,5,6,7], P. Willems[1,2,3,4], B. Wei[1,2,5,6,7], C. Tian[8], N. Bodra[1,2,5,6,7], S. Agustin Martinez Gache[5,6,7], D. Vertommen[9], K. Gevaert[3,4], K. S. Carroll[11], M. Van Montagu[1,2], J. Yang[8], F. Van Breusegem[1,2,6] and J. Messens[5,6,7]

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Hydrogen peroxide (H_2O_2) is an important messenger molecule for diverse cellular processes. H_2O_2 can oxidize protein cysteinyl thiols to sulfenic acid, a modification known as S-sulfenylation, thereby changing the conformation and functionality of the protein. Although many proteins have been identified as targets of sulfenylation in plants, site-specific mapping and quantification are lacking. By means of a peptide-centric chemoproteomic approach, we mapped 1,537 S-sulfenylated sites on over 1,000 proteins in *A. thaliana* cells under oxidative stress. We found that S-sulfenylation frequently occurs on cysteines at catalytic sites or on cysteines at metal-binding sites, hinting at a possible mode-of-action for redox regulation. When comparing the site-specific S-sulfenylation dataset of humans with *A. thaliana*, we found 155 conserved *S*-sulfenylated cysteine sites. One such site was the non-catalytic Cys181 of *Arabidopsis* MITOGEN-ACTIVATED PROTEIN KINASE 4 (AtMAPK4), which corresponds to Cys161 of human MAPK1, a cysteine reported to be redox-regulated. Replacing Cys181 of AtMAPK4 by a serine resulted in a decrease of the kinase activity, emphasizing the importance of this non-catalytic cysteine for the functionality of AtMAPK4. All in all, we deliver an unprecedented inventory of S-sulfenylated cysteines in *Arabidopsis* under oxidative stress, which could serve as an inspiration for future protein structural and functional studies.

INTEGRATED PROTEOGENOMIC, QUANTITATIVE REDOX PROTEOMIC AND METABOLOMIC APPROACHES REVEAL SIGNATURES OF SEED DORMANCY CONTROL IN WHEAT

N.V. Bykova[1], M. Jordan[1], N. Radovanovic[1], M. Rampitsch[1], J. Hu-Skrzenta[1,2], M. Huang[1]

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ROS generation, antioxidative systems, phytohormonal regulation and their reciprocal signaling were shown to play important roles in releasing embryo cells from the quiescent state during seed dormancy alleviation. Phenotype- and genotype-specific proteomic signatures of seed dormancy and association of dormancy release with ROS-mediated protein oxidation were studied using spring wheat doubled haplod populations and integration of proteogenomic, pharmacological, redox proteomic and metabolomic approaches. Dormancy-related alterations in aleurone and embryo proteomes and transcriptomes during early imbibition were studied using iTRAQ-based quantitative proteomics, mRNA-Seq differential gene expression analysis, and association with QTL regions. In dormant embryos, significant phenotype-specific changes were found for proteins involved in redox control, signaling associated with flowering, phytohormones and lipid second messengers, development and growth repression, cell cycle control and epigenetic regulation of gene expression, translational dynamics, cell wall metabolism, and ubiquitin 26S proteasome pathway. In embryos with non-dormant phenotype energy metabolism showed high capacity for the provision of NADPH reducing equivalents, pyruvate and TCA cycle intermediates for biosynthetic processes. Pathways for energy provision in non-dormant aleurone showed increased flux through the glycolytic pathway, high metabolic network flexibility, and an important role of inorganic pyrophosphate metabolism as an alternative energy donor. Inhibition of ROS production in the presence of ABA had synergistic effect on blocking germination in non-dormant seeds. Differential thiol-specific blocking followed by quantitative iodoTMT labeling-based proteomic analysis revealed sets of 813 and 535 redox responding target proteins identified in embryo and aleurone, respectively. Our results demonstrate that germination induction is mediated through the redox control, the NADPH oxidases are at least one source of that control, and wheat seeds appear to use antioxidative pathways to maintain dormancy. The total glutathione levels were significantly higher in dormant than in non-dormant and after-ripened embryos, whereas the total ascorbate levels increased upon after-ripening indicating high capacity for ascorbate regeneration.

T 11 | Wednesday, July 10, 2019, 17:00 - Session II

NEW ANAYLYTICAL METHOD ENABLING REAL-TIME H_2O_2 DETECTION IN THYLAKOIDS SHOWS THAT PSI UNIQUELY GENERATES CHLOROPLASTIC H_2O_2 SIGNAL

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Plants must regulate their biochemical processes in response to environmental stimuli and stresses including abiotic, such as light availability or drought, and biotic, such as infections by pathogens. Peroxide (H_2O_2) is considered a critical signaling molecule involved in regulating many of these responses. One complication of the peroxide signal however, is the apparent specificity of its targets in-spite of the numerous subcellular locations where it may be generated. To further complicate matters, although the chloroplast is only one of the organelles implicated in H_2O_2 signaling, multiple studies suggest that H_2O_2 may form at both PSI and the PQ pool when over reduced. Are chloroplasts able to differentiate these signals? Is over reduction of the PQ pool the same as over reduction of PSI? This leads to debate about how plants actually distinguish the signals originating from the chloroplast's thylakoid membrane, which complicates models relating back to plant signaling. Our work aimed to produce a better understanding of chloroplast H_2O_2 formation to help simplify these models. I present our findings based on a new and novel application of stable isotopes and Membrane Inlet Mass Spectrometry (MIMS) in which the formation of H_2O_2 can be directly inferred from the reaction stoichiometry of isolated thylakoid samples of *Arabidopsis thaliana*. The results unambiguously demonstrate that only electrons reaching PSI are able to participate in the formation of H_2O_2. This is further supported through a unique set of intact leaf-disc measurements that clearly demonstrate accumulation of H_2O_2 at the acceptor side of PSI and increased rates of mitochondrial respiration measured simultaneously during illumination. The results suggest the chloroplast H_2O_2 signal is a direct sensor of the equilibrium existing between reduced Ferredoxin (Fd) and $NADP^+$ at the acceptor side of PSI. Based upon this conclusion a clearer model of chloroplast H_2O_2 signaling is presented.

PITFALLS IN ACCURATE ANALYSIS OF REACTIVE CARBONYL COMPOUNDS FROM BIOLOGICAL SAMPLES

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Main objective(s): Development of a sensitive and accurate analytical procedure for quantification of reactive carbonyl compounds (RCC)

Materials and methods: GC-MS analysis of RCC as their pentafluorobenzyloximes after liquid/liquid extraction from the mostly aqueous biological samples is a frequently applied standard protocol. This protocol was challenged for accuracy (recovery) and robustness against changes in extraction conditions using methylglyoxal and glyoxal as model compounds. Moreover, since LC-MS nowadays is increasingly used to analyze RCC, we tested about 20 potentially suitable reagents to yield RCC derivatives that could be sensitively detected by LC-MS; the protocols producing the most responsive derivatives were optimized for accuracy and robustness of RCC analysis.

Results: We identified several critical issues in the established protocol for RCC quantification by GC-MS. These problems were associated with blank contamination, poor recovery and high variation of repeated measurements. We present several analytical strategies to improve the results of such analyses.
For analyses by LC-MS, we experienced similar complications. In addition, here, the most sensitive responses were unfortunately obtained from RCC derivatives of low and/or intermediate stability. We tested several approaches to improve the robustness of the protocol and applied the optimized protocol in a lipid oxidation experiment. In this application, we experienced serious matrix effects that would be expected to be inherent to any system subjected to oxidative stress.

Conclusion: As with other reactive target compounds, securing the accuracy of analysis for RCC is a sophisticated task considering the instability of such analytes during sample preparation and analysis time, a fact requiring a very careful prior consideration when RCC are to be quantified. Moreover, the ubiquitous presence of methylglyoxal and glyoxal in the environment challenges the lower calibration limits that theoretically could be achieved with sensitive protocols by strong blank contamination.

T 13 | Thursday, July 11, 2019, 9:00 - Session III

NITRIC OXIDE SYNTHASE IN PLANTS: WHERE DO WE STAND?

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Nitric oxide (NO) regulates various physiological processes in both animals and plants. In animals, NO synthesis is mainly catalysed by NO synthase (NOS) enzymes. Over the last 20 years, experimental arguments in favour of the existence of plant NOS-like enzymes have been reported but, with the exception of the green alga *Ostreococcus tauri*, no gene or protein with sequence similarity to animal NOSs has been reported in the plant genomes sequenced. Supporting this statement, recently we investigated the presence of NOS-like enzymes in over 1000 species of land plants and algae. We identified no typical NOS sequences in 1087 sequenced transcriptomes of land plants. In contrast, we identified NOS-like sequences in 15 of the 265 algal species analyzed. The algal NOSs are unequally distributed and did not correspond to phylogeny. First *in silico* and enzymatic analyses indicate that these NOS display specific structural features and probably original catalytic activities. These particularities open the possibility that algal NOSs might display particular biochemistry that remains to be investigated. Completing this picture, our recent data highlight the absence of the typical NO/cGMP signalling module mediating major NO-dependent physiological processes in animals. In contrast, they further support the importance of S-nitrosation as a main NO-dependent signalling mechanisms in plants.

[1] Jeandroz et al., Sci. Signal. 9, pp. re2, 2016
[2] Santolini et al., Nitric oxide 63, 30-38, 2017
[3] Astier et al., J. Exp. Bot., in press

T 14 | Thursday, July 11, 2019, 9:30 - Session III

COORDINATION OF CHLOROPLASTIC AND MITOCHONDRIAL ROS SIGNALING

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Plant chloroplasts and mitochondria work together to supply the cell with energy and metabolites. ROS are formed in these organelles in the electron transfer chains. Signaling from chloroplasts and mitochondria is partly dependent on ROS, which regulate many aspects of development, stress signaling, systemic responses, and programmed cell death. This communication network affects gene expression in the nucleus where numerous signals are perceived and integrated. However, the molecular mechanisms of the coordinated action of the two energy organelles in response to ROS-producing environmental cues, such as changing light intensity or pathogen responses are poorly understood. The Arabidopsis *rcd1* mutant has defects both in the mitochondria and in the chloroplasts; it has altered formation of ROS in chloroplasts, and continuously expresses the Mitochondrial Dysfunction Stimulon (MDS) genes. RCD1 serves as scaffold for nuclear protein complex formation and chloroplastic ROS affect its abundance, redox state and oligomerization. RCD1 interacts with transcriptional regulators of ROS-related mitochondrial retrograde signaling. Inactivation of *RCD1* increases expression of the MDS genes resulting in accumulation MDS gene products in the mitochondria. This affect respiration and energy metabolism, and alters electron transfer in the chloroplasts, leading to decreased chloroplastic ROS production and increased protection of photosynthetic apparatus. RCD1-dependent regulation is also involved in 3'-phosphoadenosine 5'-phosphate (PAP)-mediated retrograde signaling from chloroplasts; a significant overlap exists between genes negatively regulated by RCD1, the MDS genes, and genes affected by PAP. Sensitivity of RCD1 to organellar ROS provides feedback control of nuclear gene expression and RCD1 integrates retrograde signals from both chloroplasts and mitochondria to exert its influence on nuclear gene expression. In addition, MDS genes influence not only the mitochondria, but indirectly also the chloroplasts. Overall, RCD1 allows dialog between retrograde signals from both energy organelles. This makes it an important regulator of plant energy metabolism.

T 15 | Thursday, July 11, 2019, 10:00 - Session III

INVOLVEMENT OF THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN COMPONENTS IN NITRIC OXIDE METABOLISM IN PLANTS

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The components of the mitochondrial electron transport chain are involved not only in the reduction of oxygen as a terminal acceptor but also in the production of nitric oxide (NO) from nitrite which is facilitated during anaerobic stress. To investigate the role of the mitochondrial complex I and alternative oxidase (AOX) in NO metabolism, the wild type and transgenic tobacco (Nicotiana sp.) plants with different levels of expression of the complex I subunit NAD7 and AOX were subjected to nitrogen atmosphere and normal air. The NO emissions from plants were detected by the chemiluminescent NO analyzer, metabolite concentrations were measured by NMR analysis, and respiratory enzymes were assayed spectrophotometrically. A significant decrease in NO production was recorded under anoxia in the plants lacking complex I subunit NAD7 as compared to the wild type. The plants impaired in complex I were characterized by the elevated levels of phytoglobin even under normoxia, low level of aconitase and higher activities of the fermentation enzymes alcohol dehydrogenase and lactate dehydrogenase. The plants downregulating AOX exhibited low NO emissions and decreased levels of protein S-nitrosylation under hypoxia as compared to the plants overexpressing AOX, while under normoxia they showed higher S-nitrosylation levels. The pool size of amino acids and organic acids revealed a complex difference depending upon AOX amount under hypoxic and normoxic conditions, which was reflected in the levels of succinate, malate, glycine and γ-aminobutyric acid. AOX amount also strongly influenced the activity of aconitase under hypoxia. The level of superoxide, lipid peroxidation and total antioxidant reducing power increased in AOX-knockdown plants under normoxia and during re-oxygenation after hypoxia. It is concluded that both complex I and AOX have pervasive and oxygen concentration-dependent effects on NO production, protein S-nitrosylation, respiratory carbon and nitrogen flow, as well as on the metabolism of reactive oxygen species.

T 16 | Thursday, July 11, 2019, 10:50 - Session III

POLLEN FERTILITY AND THE ROLE OF ROS AND REDOX HOMEOSTASIS IN HEAT STRESS TOLERANCE DURING SEXUAL REPRODUCTION

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In many plants, pollen development and fertilization are considered most sensitive to heat stress (HS), which often leads to male sterility.
The ascorbate peroxidase 2 (APX2) enzyme is an important ROS/redox regulator in the HS response (HSR) in *Arabidopsis thaliana* and other plants. While *APX2* expression is male organ- and pollen-specific during reproductive development, APX2 deficient pollen gained increased thermotolerance, accounting for increased seed set under these conditions. Using our recently-developed flow cytometry approach, we show differences in pollen viability, ROS and redox status between the *apx2* mutant and WT at the population scale. This approach also demonstrated that pollen is distributed bimodally into 'low-ROS' and 'high-ROS' subpopulations, supporting a model in which a significant fraction of a flower's pollen remains in a low-metabolic or dormant state, which may serve as a stress avoidance strategy. Transcriptomics comparison between *apx2* and WT, pollen and leaves, identified nearly 1,400 *apx2* pollen-specific transcriptional changes, indicating extensive reprogramming that includes activation of the phenylpropanoids and auxin pathways. Follow-up experiments support the involvement of flavonol biosynthesis, auxin signaling, and redox regulation in the HS tolerance mechanism of *apx2* pollen. Our work provides new insights into pollen HSR, which could contribute to increasing food security in the face of the current trend of global climate change.

T 17 | Thursday, July 11, 2019, 11:10 - Session III

ARABIDOPSIS NITRIC OXIDE (NO) CONTENT IS MODULATED BY THE CHLOROPLAST MEMBRANE K^+/H^+ ANTIPORTERS, ATKEA1 AND ATKEA2

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Nitric oxide (NO) is a gasotransmitter involved in multiple physiological processes [1,2]. NO is produced in different subcellular compartments including chloroplasts, mitochondria, and peroxisomes. In a previous study, it was demonstrated that the loss of function of the **K^+ E**xchange **A**ntiporter (KEA) transporters KEA1 and KEA2, located in the inner envelope membrane of chloroplasts, provokes a reduced photosynthetic efficiency and, consequently, a reduced growth during the early development of *Arabidopsis* seedlings [3]. Using seedlings from *Arabidopsis thaliana* double knock-out (KO) AtKEA1KEA2 mutants (*Atkea1kea2*), it has been studied at biochemical level the ROS metabolism (lipid peroxidation, catalase and NADPH-generating enzymes, and reduced/oxidized glutathione) and NO content in roots and green cotyledons. The data show that the loss of function of the KEA1KEA2 chloroplast membrane does not cause alteration of the ROS homeostasis; however, the NO content was significantly affected in both photosynthetic and non-photosynthetic organs. Thus, the results suggest that the chloroplast osmotic balance and integrity maintained by AtKEA1 and AtKEA2 are necessary to keep the cellular conditions to generate NO for signaling purposes. Moreover, these data opens a new questions about how endogenous NO generation is affected by the K^+ transport located in the chloroplasts.

[1] Begara-Morales et al (2018) **J Exp Bot** 69: 3425–3438
[2] Astier et al. (2018) **J Exp Bot** 69: 3401-3411
[3] Aranda-Sicilia MN et al., (2016) **Plant Physiol** 172:441-449.

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T 18 | Thursday, July 11, 2019, 11:30 - Session III

ERO-MEDIATED THIOL OXIDATION IS ESSENTIAL FOR PLANT ER REDOX HOMEOSTASIS AND ETHYLENE SIGNALLING

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The plant endomembrane system is particularly rich in disulfide-containing proteins including storage proteins, secreted proteins and membrane-resident proteins. *De novo* formation and isomerization of disulfides, referred to as oxidative folding, requires specific thiol oxidoreductases and thiol oxidases. In the secretory pathway, two thiol oxidases, quiescin sulfhydryl oxidases and ER thiol oxidases (ERO) exist with two isoforms each. Here we addressed the function of EROs in maintaining oxidizing conditions in the ER and its implications for physiological functions. Production of H_2O_2 as a by-product requires suitable detoxification machineries for H_2O_2 which are not yet known. Arabidopsis null mutants and RNAi knockdowns were employed to address the contribution of both EROs in ER redox homeostasis. For direct observation of the thiol redox status in the lumen, the glutathione redox potential (E_{GSH}) was measured as a proxy. To enable dynamic measurements of E_{GSH}, we used roGFP2-iL-Grx1 which has a less negative midpoint potential than the parent roGFP2. In addition, roGFP2 was used in protein fusions to identify and localize novel proteins that may be involved in maintaining ER redox homeostasis. Diminished ERO activity results in severe sensitivity towards DTT displayed by dwarf phenotypes. Expression of roGFP2-iL-Grx1 in the ER showed that diminished ERO activity leads to shift of the local E_{GSH} towards less oxidising conditions and dynamic measurements revealed a delayed oxidation capacity. By expressing roGFP2 fusion proteins we identified glutathione peroxidase-like 3 (GPXL3) as a type II membrane-bound protein resident in the early secretory pathway and thus as a first candidate for local H_2O_2 degradation. Further investigation of *ero* mutants under non-stress conditions revealed a pronounced insensitivity towards ethylene which identifies the ethylene receptor ETR1 as a sensitive target of ERO activity. From our observations we deduced a model for integration of ER thiol oxidation processes with cytosolic phytohormone signalling.

T 19 | Thursday, July 11, 2019, 14:00 - Session IV

ELEVATED TEMPERATURES AND DROUGHT DURING SEED MATURATION AFFECT REDOX SIGNALING

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Seeds are pivotal to agricultural productivity and food security, yet there remain substantial gaps in our understanding of the critical role that the environment plays during seed development and storage, and its effect on seed quality. Here we report on the effects of abiotic stress factors experienced by the mother plant on seed quality, with a focus on redox signalling, viewed through a multi-omics approach in combination with targeted HPLC analyses of antioxidants. *Arabidopsis thaliana*, *Brassica oleracea*, *Hordeum vulgare* and *Helianthus annuus* plants were subjected to suboptimal temperatures and drought, close to conditions predicted in climate change scenarios. Amongst other effects, elevated temperatures led to reduced thermodormancy in *A. thaliana* and *H. vulgare*, and affected mean seed size and quality in *A. thaliana* and *B. oleracea*. In all species, the "maternal environment" clearly affected the concentrations and redox state of antioxidants such as glutathione (g-glutamyl -cysteinyl-glycine), concentrations of tocochromanols and protein carbonylation, thereby resetting the seeds' redox environment as well as reprogramming their genomes. Gene ontology analysis confirmed that pathways involved in redox signalling were affected by the abiotic stress factors experienced during seed development, with downstream regulation of important seed quality traits such as seed viability, dormancy and longevity.

T 20 | Thursday, July 11, 2019, 14:30 - Session IV

MEDIATOR AND ELONGATOR SUBUNITS REGULATE H_2O_2 SIGNALING AND RESPONSES TO OXIDATIVE STRESS

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Hydrogen peroxide (H_2O_2) is the most predominant ROS produced during photorespiration and is able to trigger extensive transcriptional reprogramming necessary for subsequent defense responses. To identify proteins that sense H_2O_2, transduce and mediate signaling, we have designed several genetic screens. In these, we identified a subunit of the Mediator complex (transcription initiation), MED8, as a negative regulator of early H_2O_2 -responsive gene expression in *Arabidopsis thaliana*. T-DNA lines harboring insertion in the N-terminal domain of MED8 were embryo-lethal, suggesting the crucial role of MED8 in development. Interestingly, mutants with deletion of the C-terminal glutamine (Q)-rich domain (*med8ΔQ*) are viable, and display increased tolerance to oxidative stress. When *med8ΔQ* mutation was introduced into the *catalase-deficient* (*cat2*) mutant, the *cat2 med8* double mutants displayed increased lesion formation, activation of salicylic acid pathway and the induction of pathogenesis-related gene expression in long days. Moreover, *med8ΔQ* mutation trigger lesions development in short days, condition otherwise non-permissive for lesions development. In a second screen, we have exploited the conditional nature of *cat2* to identify secondary mutations that modulate the cell death phenotype. The Elongator subunits (transcription elongation) ELP1 and ELP2 have been isolated in the screen and their effects on the H_2O_2 driven cell death have been validated using T-DNA knockout mutants. The impact of *elp1* and *elp2* on redox homeostasis, salicylic acid accumulation and gene expression was analyzed in both Col-0 and *cat2* backgrounds. Preliminary data suggest that *elp1 and elp2* mutations suppress of the cell death phenotype associated with down regulation of the salicylic acid pathways and reduced acetylation marks at several loci. In summary, we report a novel molecular link between the Mediator and the Elongator complexes and H_2O_2 -signaling and -dependent gene expression.

T 21 | Thursday, July 11, 2019, 15:00 - Session IV

SINGLET OXYGEN MEDIATED STRESS RESPONSES ARE GOVERNED BY RNA OXIDATION AND ATTENUATION OF CELLULAR TRANSLATION

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The generation of singlet oxygen ($^1O_2$) in cells occurs during various stresses. $^1O_2$ readily oxidizes guanylate nucleotides in nucleic acids to form 8-hydroxyguanosine in RNA (8-oxoG), but the consequence of 8-oxoG accumulation in the cell is poorly understood. Rose bengal, a photodynamic agent was used to generate $^1O_2$ in Arabidopsis seedlings. The increased presence of 8-oxoG in RNA affected a decrease in the translatability of mRNA *in vivo* and in a reticulocyte system. Transcripts induced photodynamically by rose bengal demonstrate extensive overlap with transcripts stimulated by the translation inhibitor cycloheximide. The results present a scenario whereby $^1O_2$ causes direct oxidation of cellular mRNA decreasing its translatability to the extent where specific genes are released from the repression of their cognate short half-life repressors. Indeed, jasmonate-responsive genes known to be regulated by SCF^{COI1} ubiquitin ligase-dependent degradation of JAZ1 repressor protein were shown to be induced after $^1O_2$-induced attenuation of cellular translation in a SCF^{COI1}-independent manner. Similarly, drying stress in high light resulted in RNA oxidation and SCF^{COI1}-independent induction of these transcripts. The findings provide a mechanism for direct effects of the short-lived $^1O_2$ on cellular metabolism and explain the origin of common stress transcriptomes promoted by diverse $^1O_2$-dependent processes.

T 22 | Thursday, July 11, 2019, 15:50 - Session IV

FAST REDOX RESPONSE OF IRON-SULFUR GLUTAREDOXIN GRXS17 ACTIVATES ITS HOLDASE ACTIVITY AND PROTECTS PLANTS FROM HEAT STRESS

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Arabidopsis GRXS17 is an iron-sulfur clusters (ISC) glutaredoxin that consists of an N-terminal TRX-domain and three CGFS-active site motif-containing GRX-domains, coordinating three ISC in a glutathione (GSH)-dependent manner. As an ISC-charged holoenzyme, GRXS17 is likely involved in the maturation process of ISC-containing proteins. In addition of its role in cluster biogenesis, we showed here that GRXS17 is a foldase with redox-dependent holdase activity. Oxidative stress in combination with heat stress induces loss of the ISC followed by sulfenylation of active site cysteines and subsequent formation of disulfide bonds between conserved cysteines in the corresponding N-terminal TRX domains. This oxidation leads to the activation of the holdase activity, and shifts GRXS17 from a low-MW form to a high-MW complex. Furthermore, we showed that GRXS17 changes its client proteins under heat stress, and protects them from aggregation. All in all, we reveal the mechanism of an ISC-dependent activity shift, turning the holoenzyme GRXS17 into a holdase that prevents damage under heat stress.

T 23 | Thursday, July 11, 2019, 16:10 - Session IV

THE MKKK70-MKK4-MPK3 CASCADE AND LORD1 MODULATE ROS-DEPENDENT SERF1 TRANSCRIPTION FACTOR ACTIVITY TO COORDINATE THE INITIAL RESPONSE TO SALT STRESS IN RICE

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Salinity is one of the most prominent abiotic factors that threatens food security worldwide. We previously discovered a novel reactive oxygen species (ROS) dependent pathway in rice that provokes transcriptional responses to mediate salt stress adaptation. At the heart of this network acts the transcriptional regulator SALT-RESPONSIVE ERF1 (SERF1), whose transcription and activity is induced within the first minutes of exposure to salt stress.

Here, we provide genetic evidence that SERF1 acts downstream of a novel MITOGEN-ACTIVATED PROTEIN KINASE (MPK) cascade, of which each component can stimulate SERF1 activity independently. Furthermore, with *in planta* inhibitor experiments, we expose NADPH oxidases as the major ROS source required for activation of the SERF1-dependent transcriptional cascade. SERF1 shows an exquisite spatial and temporal activity that is limited to the initial hour after exposure to salt stress. To understand the temporal dynamics of SERF1 activity, we explored the underlying mechanism regulating transcription factor inactivation. Through a comprehensive yeast-two-hybrid screen, we identified LATENT OPPOSER OF DREB1 (OsLORD1), an uncharacterized nuclear zinc-finger protein, which physically interacts with the SERF1 DNA binding domain. Interaction between SERF1 and OsLORD1 results in loss of transcriptional activity, thereby mechanistically contributing to SERF1 inactivation. Notably, *OsLORD1* expression under salt stress is regulated in a ROS-dependent manner, however, its induction occurs independently from the SERF1 signaling module.

We propose that the precise timing of activating ROS-dependent signals involving MPKs and induction of inhibiting proteins both controls SERF1 function and allows rapid and balanced initiation of salt signaling leading to stress adaptation. Furthermore, our results suggest early branching of the initial ROS signal during salt stress, revealing an exciting novel concept in ROS signaling.

T 24 | Thursday, July 11, 2019, 16:30 - Session IV

HYDROGEN PEROXIDE- AND REDOX-MEDIATED SIGNALLING: WHERE WE ARE NOW OR HISTORY IS BUNK

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It is arguable that the starting point for research in the plant sciences on hydrogen peroxide (H_2O_2)-and redox-mediated signalling was born out of addressing the biochemical and physiological basis of oxidative stress, 20+ years ago. Perhaps many of our observations and views have not progressed dramatically since those early days. The 'omics revolution, the advent of powerful computational biology, the ease of making transgenic plants and mutants, at least in model species, have all been done with an eye on extending our understanding of redox- and H_2O_2-mediated signalling. The important contribution this work has achieved is really in emphasising the extent and scale of redox- and H_2O_2-mediated signalling in many aspects of plants' lives. This history also illustrates that to make progress the field has been heavily dependent on technological advances rather than developing new concepts and hypotheses to further our understanding.

That's the history, what about the present? New technologies have emerged to be used alongside those we have been using for a decade or more. I will describe those technologies, which I think could at least pose challenging questions and which would be the starting point in the development of new concepts. I am optimistic about the future of this research, there is much for our younger colleagues to do, if they do not take too much notice of history.

In summary, I will try to peer into the future and to give a personal view of some technologies which could really open up the way to new signalling concepts and what these might be. As with fortune, the future will favour the brave.

T 25 | Friday, July 12, 2019, 09:00 - Session V

ADAPTATION TO ENVIRONMENTAL STRESS BY A DYNAMIC CHROMATIN-BASED STRESS MEMORY

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In nature, plants often encounter chronic or recurring stressful conditions. An increasing number of observations suggest that plants can be primed by exposure to stress, thereby activating a stress memory that enables a more efficient response upon a recurring stress incident. My lab studies heat stress memory in plants as a model case for environmental stress memory. Seedlings acquire thermotolerance through a heat treatment at sublethal temperatures (priming heat stress) that enables them to survive an otherwise lethal heat stress. This thermotolerance is actively maintained for several days as indicated by the existence of mutants which are able to establish thermotolerance, but fail to maintain it.
We have previously found that heat stress induces sustained histone methylation at heat stress memory-related loci that outlasts the transcriptional activity of these loci and marks them as recently transcriptionally active. In a forward genetics approach, we have found that regulation of nucleosome occupancy is also required for sustained activation of memory gene expression. Sustained low nucleosome occupancy is mediated by the FORGETTER1 (FGT1) protein through interaction with chromatin remodeling proteins. From the same genetic screen, we have identified additional components that positively regulate HS memory and indicate further involvement of transcriptional regulation, but also a crosstalk with membrane dynamics. In summary, the physiologically defined phenomenon of HS memory has a molecular equivalent in the transcriptional memory and associated changes in chromatin structure and membrane dynamics.

LSD1, EDS1 AND PAD4 INVOLVEMENT IN ROS REGULATION AND STRESS RESPONSE IN PLANTS

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In their natural environment, plants are continuously exposed to a broad range of stress, which leads to a disturbance of cellular homeostasis, and can trigger cell death. Reactive oxygen species (*ROS*) are one of the most important molecules involved in the induction, signaling, and execution of *plant cell death.* Therefore, mutants with deregulated ROS accumulation constitute a good model for studying cell death mechanisms.

In *Arabidopsis thaliana*, the mutation in *LESION SIMULATING DISEASE 1* (*LSD1*) was originally associated with superoxide-dependent cell death, caused by biotic stresses. The phenotype of *lsd1* mutant is characterized by the so-called runaway cell death (RCD), manifesting itself in fast propagation of lesions, which gives the genetic evidence for LSD1 as a cell death repressor. Later, it was shown that uncontrolled spread of foliar RCD in *lsd1* is also associated with accumulation of hydrogen peroxide, salicylic acid as well as ethylene, and can be evoked by many abiotic factors. Interestingly, during combined drought and high-light stress or in natural, field conditions, *lsd1* proves to be more tolerant than wild-type plants.

Importantly, cell death in *lsd1* mutant depends on two proteins that were originally described as components of basal disease resistance, ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4). LSD1 together with EDS1 and PAD4 play an important role not only in regulation of ROS and hormonal homeostasis, but also photosynthetic efficiency, water use efficiency and seed yield. In fact, the LSD1/EDS1/PAD4 -dependent correlation among salicylic acid, hydrogen peroxide, water use efficiency, and seed yield can be described by mathematical equations, and can be the starting point in the creation of an algorithm that would allow to estimate the seed yield at the initial stage of plant growth, based on some molecular and physiological traits.

Our recent results demonstrate that LSD1, EDS1 and PAD4 show common nucleo-cytoplasmic localization. Furthermore, LSD1 forms homodimers and interacts with EDS1 and the broad range of other proteins engaged in various molecular pathways. Importantly, the interaction of LSD1 with its partners is dependent on plant redox status. We also showed that additionally to its scaffold protein function, LSD1 acts as a transcriptional regulator.

Moreover, we proved that *Populus tremula L. x tremuloides* ortologs of EDS1 and PAD4 play a role in ROS metabolism regulation and together with LSD1 alter wood tissue composition. All these results demonstrate that LSD1/EDS1/PAD4 constitute a molecular hub for ROS and hormonal homeostasis regulation in response to stress.

T 27 | Friday, July 12, 2019, 10:00 - Session V

PLANT PATHOGENS HIJACK HOST REDOX INTERMEDIATES TO SUPPRESS IMMUNE SIGNALLING NETWORKS

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Both oxidative and reductive bursts have been associated with the fine tuning of immune hormonal signalling in plants. Reactive thiol groups in proteins act as redox switches that sense the chemical environment by being subjected to a number of distinct redox-based, post-translational modifications. Particularly, S-nitrosylation, the covalent attachment of a nitric oxide (NO) moiety to reactive thiol groups to form a protein-SNO, has been shown be extensively implicated in immune responses by altering protein dynamics. Appropriate protein-SNO signalling is thought to be achieved by coordination between NO synthesis and scavenging, as well as protein-SNO formation and selective reduction. Recently, our group proposed that distinct protein-SNO branches determine fate and amplitude of immune responses by targeting different transcriptional programmes. Molecular mechanisms employed to drift between different protein-SNO branches, however, remain largely elusive. Here we provide genetic and biochemical evidence that protein-SNO signal propagation in plants is hijacked by pathogens during onset of plant immune responses to promote virulence. Our work indicates that a bacterial-derived virulence factor targets specific protein-SNO branches in an unexpected fashion to wear down the host immune system. We propose a model in which the outcome of plant-pathogen interactions is determined by a molecular battle between hosts and adapted pathogens to offset the equilibrium of interchangeable reactive nitrogen intermediates that shape protein-SNO signalling branches.

T 28 | Friday, July 12, 2019, 10:50 - Session V

REACTIVE OXYGEN SPECIES CONTRIBUTE TO THE SYMPTOMLESS, EXTREME RESISTANCE TO *POTATO VIRUS X* IN TOBACCO

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Rx resistance genes confer symptomless, extreme resistance (ER) to *Potato virus X* (PVX). During ER the lack of localized cell/tissue death (hypersensitive reaction, HR) is likely due to a rapid, early inhibition of virus replication (Bendahmane et al., 1999, Plant Cell 11, 781). However, the mechanism(s) of virus inhibition during ER is largely unknown. Our main aim was to clarify the possible contribution of reactive oxygen species (superoxide, $O_2^{\cdot-}$; hydroxyl radical, $OH^{\cdot}$) in the ER to PVX conferred by the *Rx*1 resistance gene in tobacco (*Nicotiana tabacum*). Tobacco lines were used that carry *Rx*1 from potato or overexpress a ferritin gene from alfalfa (tobacco cv. Samsun NN, Rx; cv. SR1, C8 and F9). PVX-accumulation was monitored by real time RT-qPCR. Superoxide was detected by nitro blue tetrazolium (NBT) staining. Treatments with antioxidants (superoxide dismutase, SOD; catalase, CAT) and riboflavin/methionine were conducted by injecting PVX-inoculated leaves (Hafez et al., 2012, Phytopathology 102, 848).
In "Rx" plants, superoxide accumulation was detectable early, within six hours after PVX-inoculation, virus titers sharply decreased only after this time point, while in susceptible tobacco superoxide levels were markedly lower. Superoxide inhibition by antioxidants (SOD, CAT) partially suppressed ER: PVX titers significantly increased and HR-like symptoms developed. F_1 progeny from crosses of "Rx" to ferritin-overproducer ($OH^{\cdot}$-deficient) tobaccos also displayed a suppressed ER; virus levels significantly higher than in "Rx" plants were coupled to HR. Furthermore, treatment of PVX-susceptible tobacco with a superoxide-generating agent (riboflavin/methionine) resulted in HR-like symptoms and reduced PVX-titers, supporting the possible role of superoxide in ER. Our results demonstrate the contribution of early accumulation of ROS ($O_2^{\cdot-}$, $OH^{\cdot}$) in limiting PVX replication during symptomless ER. Further research should characterize the exact role(s) of various ROS in the signal transduction of symptomless, extreme virus resistance.

This research was supported by grants of NKFIH-OTKA (K111995, K128868).

T 29 | Friday, July 12, 2019, 11:10 - Session V

THIOREDOXINS CONTROL SPECIFIC NITRIC OXIDE SIGNALLING BRANCHES IN PLANT IMMUNITY

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Cellular redox changes play important roles in eukaryotic immune responses. Upon pathogen attack, host cells produce large quantities of reactive oxygen and nitrogen species. Particularly, the redox active signalling molecule nitric oxide (NO) plays important roles in regulating processes ranging from development to responses to (a)biotic stresses. NO relays information mainly through *S*-nitrosylation, *i.e.* the covalent modification of reactive cysteine residues with an NO moiety to form *S*-nitrosothiols (protein-SNO). *S*-nitrosylation has emerged as a key mechanism to control the activity, conformation, protein-protein interaction and localisation of a growing number of proteins. In plants, excessive protein-SNO accumulation has been associated with disease susceptibility, suggesting that cellular mechanisms of protein-SNO removal are crucial to effective immune responses. Recently, it was shown that the evolutionary conserved oxidoreductase, Thioredoxin-*h*5 (TRX*h*5), controls protein-SNO levels by acting as a direct and selective protein-SNO reductase. Genetic evidence indicates that TRX*h*5 only reduces protein-SNO derived from modification by free NO, but not by the potent natural NO donor *S*-nitrosoglutathione (GSNO). By using genetic and biochemical tools, here we provide evidence that the pathogen-inducible TRX superfamily member, Nucleoredoxin 1 (NRX1), selectively rescued the immune-compromised phenotype of mutants containing elevated levels of GSNO. Accordingly, overexpression of NRX1 partially restored gene expression programs induced by the immune hormone salicylic acid and exhibited unique protein-SNO reductase activity *in vitro*. Finally, based on the known involvement of TGA transcription factors in immune gene expression, we evaluated TGA susceptibility to *S*-nitrosylation and the ability of thioredoxins to reverse this modification. Collectively, our findings demonstrate that TRX*h*5 and NRX1 regulate distinct subsets of protein-SNO during plant immunity and provide a framework for dissection of further, as yet unknown, NO signalling branches during plant development and stress trade-off.

Part of this research was funded by grants BIO2017-85758-R and CSD2007-00057 (TRANSPLANTA) from the Ministerio de Ciencia, Innovación y Universidades (Spain), and SA093U16 and SA313P18 from Junta de Castilla y León (to O.L.). M.G. F.-E. is supported by a predoctoral fellowship awarded by Junta de Castilla y León.

T 30 | Friday, July 12, 2019, 11:30 - Session V

THE RESPONSE OF UNCOUPLING PROTEINS TO BACTERIAL ELICITOR INDUCED OXIDATIVE BURST

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Main objective of the study: Plant UCPs are proved to take part in the fine-tuning of mitochondrial ROS generation. It has emerged that mitochondrion can be an important early source of intracellular ROS during plant -pathogen interaction thus plant UCPs must also play key role in this redox fine-tuning during the early phase of plant–pathogen interaction. On the contrary of this well-established assumption, the expression of plant UCPs and their activity has not been investigated in elicitor induced oxidative burst. Thus, the level of plant UCPs both at RNA and protein level and their activity was investigated and compared to AOX as a reference in *Arabidopsis thaliana* cells due to bacterial harpin treatments.

Materials and methods: The expression at RNA and protein level and the activity of plant UCP was investigated in *Arabidopsis thaliana* cell cultures treated by harpin protein from *Pseudomonas syringae* pv. tomato DC3000 ($HrpZ_{pto}$)

Results: Similar to the expression and activity of AOX, the transcript level of *UCP4, UCP5* and the UCP activity increased due to harpin treatment and the consequential oxidative burst. The expression of *UCP4* and *UCP5* elevated 15-18-fold after 1 h of treatment, then the activity of UCP reached its maximal value at 4h of treatment.

Conclusion: The quite rapid activation of UCP due to harpin treatment gives another possibility to fine tune the redox balance of plant cell, furthermore explains the earlier observed rapid decrease of mitochondrial membrane potential and consequent decrease of ATP synthesis after harpin treatment.

T 31 | Friday, July 12, 2019, 11:50 - Session V

THE 2-HYDROXY CARBOXYLIC ACID ISOLEUCIC ACID MODULATES DEFENSE AND GROWTH IN *ARABIDOPSIS THALIANA*

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Plants contain branched-chain amino acid-related (BCAA) 2-hydroxy carboxylic acids. Among them, isoleucic acid (ILA, 2-hydroxy-3-methylpentanoic acid) occurs ubiquitously in plants, while valic acid and leucic acid were sporadically found. ILA has been shown to impact plant defense response in *A. thaliana*; it can be glucosylated by the small-molecule glucosyltransferase UGT76B1. Here, we examined in detail the impact of ILA on Arabidopsis defense and growth, which revealed three independent and specific reactions distinct from related compounds.

ILA application induced endogenous salicylic acid (SA) content and resistance towards *Pseudomonas syringae*. ILA synergistically activated SA-responsive gene expression and resistance in a UGT76B1-dependent manner. These observations are in line with an ILA-dependent repression of SA glucosylation and, hence, activation of the SA response. However, ILA also showed an SA-independent stress response. Superoxide was induced by enhanced exogenous or endogenous ILA in wild type and in *sid2 nahG* lines, indicating the independence of this response from SA. Since SA and ROS interact, it cannot be excluded that the superoxide induction is impacting on pathogen defense in the wild type. Furthermore, ILA showed a developmental effect by inhibiting root growth, which could be separated from the above responses. It was SA-independent and not related to superoxide induction.

The effects of ILA are specific and distinct from its isomeric compound leucic acid (LA) and from the amino acid isoleucine. LA and Ile did neither show an impact on *PR1* induction nor on ROS production, while both compounds inhibited root growth. The conservation of the complete action profile of ILA at least in crucifers was demonstrated with *Brassica napus*; ILA enhanced *PR1* expression, superoxide induction and root growth inhibition.

T 32 | Friday, July 12, 2019, 13:15 - Session VI

MITOCHONDRIAL MTERF PROTEINS AND STRESS TOLERANCE

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Mitochondria play critical roles in plants not only as essential organelles for ATP generation, but also in responding to abiotic stress by influencing nuclear gene expression. Further, mitochondria can contribute to stress by over-producing reactive oxygen species (ROS). Mutations in several mTERF (Mitochondrial Transcription tERmination Factor-related) proteins in *Arabidopsis thaliana* have been shown to affect abiotic stress responses in plants. We identified a mutation in Arabidopsis *MTERF18*/*SHOT1* (*Suppressor of hot1-4 1*) that enables plants to better tolerate heat and oxidative stresses, presumably due to low ROS levels and reduced oxidative damage. To understand the molecular mechanism by which a defect in SHOT1 reduces ROS and enhances heat stress tolerance, we are defining molecular defects of *shot1* mutants and targets of the SHOT1 protein. We found that *shot1* mutants have problems in assembly of oxidative phosphorylation (OXPHOS) complexes, and high levels of alternative electron transport chain components, which likely contributes to reduced ROS in the mutant. Immunoprecipitation of DNA followed by sequencing revealed that SHOT1 binds to specific mitochondrial DNA sequences. ATAD3 proteins, which are suggested to be involved in nucleoid organization in animals, were identified as interacting with SHOT1. With evidence of diffuse mitochondrial nucleoids in the *shot1-2* mutant, we suggest that SHOT1 may be involved in mitochondrial nucleoid organization, possibly tethering mitochondrial DNA to membrane-localized ATAD3 proteins. Our data also suggest that proper nucleoid organization is critical for assembly of mitochondria-encoded subunits of OXPHOS complexes. We are also investigating the roles of other mitochondrial-localized mTERF proteins.

T 33 | Friday, July 12, 2019, 13:40 - Session VI

REACTIVE CARBONYL SPECIES (RCS) METABOLISM AND SIGNALING IN GLYCOPHYTIC MODEL *ARABIDOPSIS THALIANA* AND HALOPHYTIC MODEL *EUTREMA PARVULUM*

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Break-down of lipids in cell membranes by ROS causes production of lipid peroxidation products such as 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE), and acrolein (ACR) which are called as reactive carbonyl species (RCS). Although there are studies about RCS in animals, studies in plants are limited, which mostly investigate their detoxification mechanisms. The aim of this work was to understand RCS metabolism and its signalling comparatively in glycophyte *Arabidopsis thaliana* and halophyte *Eutrema parvulum*, which is important for elucidation of new mechanisms related to salt stress tolerance. RCS profiles of *A. thaliana* and *E. parvulum* were determined under salinity (50 mM-600 mM NaCl) and also the activities of RCS detoxification enzymes aldehyde dehydrogenase (ALDH), alkenal reductase (AER), aldo-keto reductase (AKR), glutathione-S-transferase (GST) were measured. The effects of exogenous RCS (HNE, HHE, ACR) on the activities of RCS detoxification enzymes (ALDH, AER, AKR, GST) and the activities of antioxidant defence system were also elucidated. ROS signalling also cross-talk stress hormones such as ABA, JA, SA under stress. Therefore, the effects of exogenous RCS on contents of ABA, JA and SA were determined. Morevoer, effects of RCS on expression of ion transporters were also investigated. Data provided evidence for regulation of antioxidant defence enzymes and ROS signalling in plants depending on the type and concentration of the RCS. In regards of glycophyte and halophyte comparison, remarkable differences were, (i) response of *A. thaliana* H_2O_2 scavenging enzymes (CAT, POX, APX) was stronger to RCS treatment (ii) NADPH oxidase mediated ROS signalling was downregulated in *A. thaliana* in response to RCS. In addition, gene expression data demonstrated that RCS treatments induced *SOS1*, *NHX1* and *NHX5* expression depending on type and concentration of RCS. Moreover, growth data indicate that RCS treatments can mitigate negative effects of salt stress in *E. parvulum.*

T 34 | Friday, July 12, 2019, 14:05 - Session VI

ALDEHYDE OXIDASE 3 THAT CATALYZES THE LAST STEP OF ABA BIOSYNTHESIS ACTS AS A REACTIVE CARBONYL ALDEHYDES DETOXIFIER

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Aldehyde Oxidases (AOs) are molybdenum hydroxylases that catalyze aromatic and aliphatic aldehydes to their corresponding carboxylic acids, including oxidation of abscisic aldehyde to abscisic acid (ABA). In plants, environmental stresses result in oxidative stress, lipid peroxidation and the generation of reactive carbonyl species. Multiple enzymes contribute to the detoxification of the toxic aldehydes amongst aldehyde dehydrogenase, aldehyde reductase, aldo-keto reductase and 2-alkenal reductase, yet a role for plant aldehyde oxidases was rarely shown. Examining the sensitivity to toxic aldehydes such as acrolein, crotonaldehyde, 3-Z-hexanal and hexanal, revealed higher resistance of the Arabidopsis wild-type (WT) plants as compared to 2 independent aldehyde oxidase 3 knock out (*Ataao3 KO*) mutants. Importantly, the internal level of aldehydes such as acrolein, benzaldehyde, propionaldehyde, nonenal, trans-2-nonenal, HNE, hexanal was higher in the mutants than in WT, indicating a roll for AAO3 in detoxifying aldehydes. Furthermore, plants grown in plates (to diminish any drought effect) exhibited earlier senescence symptoms in the mutants, likely the result of the accumulation of higher carbonyl aldehydes such as acrolein, crotonaldehyde, 3-Z-hexanal and hexanal. Additionally, ultraviolet C irradiation induced early premature senescence accompanying by higher level of carbonyl aldehydes and loss of chlorophyll content in leaves of KO mutants as compared with WT, whereas no differences in relative water content was evident. Altogether, our results indicate that AAO3 is playing an important role in detoxifying reactive carbonyl aldehydes.

T 35 | Friday, July 12, 2019, 14:25 - Session VI

FUNCTION AND REGULATION OF THE PLASTID PEROXIREDOXIN II E

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Under optimal growth conditions, reactive oxygen species (ROS) function as signaling molecules in the cell. Most biotic and abiotic stressors cause an increase of the ROS content which may even reach toxic levels. An enzymatic antioxidant defense system exists to detoxify the harmful ROS. Peroxiredoxins (Prx) are part of this system and are divided into four classes in plants, namely 2-CysPrx, 1-CysPrx, type II Prx and PrxQ. The classification is based on sequence similarities, the location of the peroxidatic and resolving cysteinyl residue and the electron donors. Peroxiredoxin II E (PrxIIE, At3g52960) belongs to the type II Prx group and is localized in the chloroplast stroma.

Here we present data on the activity and regulation of the plastid PrxIIE. By mass spectrometry and immunoreaction, it is shown that the peroxidatic Cys121 is subjected to S-glutathionylation. This posttranslational modification of PrxIIE results in a lower peroxidase activity and may also have an impact on signaling functions. The potential role of PrxIIE in signaling was addressed by a search for proteins interacting with PrxIIE. Therefore, an affinity chromatography with immobilized PrxIIE was performed for protein selection. Bound and eluted proteins were trypsinated, peptides separated by nanoliquid chromatography and addressed by mass spectrometric analysis. From the 19 identified chloroplast proteins a 14-3-3 protein was selected for further scrutiny. 14-3-3 proteins are known to preferentially interact with phosphorylated proteins, therefore PrxIIE phosphomimic variants were generated. An overlay assay showed an inhibitory effect of phosphorylation on the interaction which also is subjected to redox regulation. The stimulated interaction under oxidizing conditions suggests that PrxIIE fulfils a novel function as redox sensor besides its thiol peroxidase activity.

T 36 | Friday, July 12, 2019, 14:45 - Session VI

THE MOONLIGHTING FUNCTION OF SUPEROXIDE DISMUTASE DEPENDS ON A NOVEL CLASS OF TRANSCRIPTIONAL CO-ACTIVATORS

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Superoxide dismutase (SOD) has been regarded for more than 50 years as an important, pivotal antioxidant enzyme. However, recent work in yeast and humans indicates that copper-zinc SOD affects transcription inside the nucleus. Here we show that superoxide dismutase 1 (CSD1) from *Arabidopsis* and rice is a moonlighting protein. Next to its common scavenging function, we reveal that CSD1 acts as a transcriptional regulator in the nucleus. Through fluorescent recovery after photobleaching (FRAP) experiments, we found that CSD1 translocates to the nucleus upon stress. In addition, by using a SELEX approach we revealed that CSD1 is a DNA-binding protein that recognizes a specific DNA motif. Moreover, we are using DNA affinity purification sequencing (DAP-seq) to uncover the genome-wide binding landscape of CSD1 in Arabidopsis. Interestingly, inside the nucleus, CSD1 interacts with a member of the so far poorly characterized HEAVY-METAL-ASSOCIATED ISOPRENYLATED PLANT PROTEIN (HIPP) family. Our data demonstrate that HIPP proteins are a novel class of conserved transcriptional co-activators both in Arabidopsis and rice. The rapid translocation and transcriptional complex formation with HIPP proteins enables CSD1 to be an important early player in a so far unknown redox-sensing mechanism. Exploring and understanding the moonlighting features of CSD1 will have major impacts on fundamental biology and potentially results in novel breeding strategies for crops.

Abstracts
Posters

Elevator Pitch I - Wednesday, July 10, 2019

Elevator Pitch II - Thursday, July 11, 2019

Posters

P 1

THE ROLE OF REACTIVE OXYGEN SPECIES IN RECEPTOR-LIKE KINASE SIGNALING AND *VICE VERSA*

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Biotic and abiotic stresses induce reactive oxygen species (ROS) production in plants as a signalling strategy. The receptor-like protein kinases (RLKs) are largely responsible for communication between cells and the extracellular environment, and ROS production is a frequent result of RLK signalling in a multitude of cellular processes. However, many of the components for extracellular ROS perception, signal transmission, and specificity of downstream responses remain unknown. Cysteine-rich receptor-like kinases (CRKs) represent a subgroup of RLKs, defined by a conserved pattern of cysteines in their extracellular domain. Based on their expression profile and loss-of-function phenotypes CRKs are promising candidates to be involved in ROS signalling. While due to their cysteine-rich extracellular domain CRKs have been considered to be potential ROS sensors, our research reveals a high similarity to fungal lectin domains suggesting that CRKs and related proteins may not sense ROS but rather small extracellular molecules.

We have identified several CRKs including, as essential integrators of signal transduction at the plasma membrane in response to biotic and abiotic stress. CRKs concert ROS production as well as calcium signaling in response to a stimulus. CRKs can interact with NADPH oxidases directly and control their activity through a novel mechanism in heterologous systems and in plants. This regulatory mechanism is likely conserved for the majority of plant RBOH proteins and shares significant similarity with the regulation of animal NOX proteins. CRKs furthermore convey downstream effects such as callose deposition synergistically with ROS and Ca^{2+}.

The genomes of higher plants encode a large number of CRK genes; however, expansion of different subtypes of CRKs has happened very differently in various plant lineages. This variation is an interesting tool for studying the origin and evolution of large protein families. It however also highlights the difficulties in translating results from model species to crop. A combination of physiological, biochemical and evolutionary/genomic approaches using the CRKs could pave the way for future understanding of large families of receptors or other protein families in plants.

P 2

NADPH OXIDASE-MEDIATED "ROS-Ca^{2+} HUB" IN SIGNALLING AND ADAPTATION TO SALINITY AND FLOODING

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Plant adaptation to salinity and flooding relies on elevation in the cytosolic free calcium. In this work, I focus on mechanisms responsible for shaping stress-specific cytosolic Ca^{2+} "signatures" and the role of NADPH oxidase in this process. I show that calcium-activated NADPH oxidases and reactive oxygen species (ROS)-activated Ca^{2+} conductances form a self-amplifying 'ROS-Ca^{2+} hub', enhancing and transducing Ca^{2+} and redox signals. The ROS-Ca^{2+} hub contributes to physiological reactions controlled by ROS and Ca^{2+}, demonstrating synergism and unity of Ca^{2+} and ROS signalling mechanisms. I then illustrate the physiological significance of ROS-Ca^{2+} hub operation in plant adaptive responses to hypoxia (flooding) and salinity. In Arabidopsis, expression of *RBOHD* was down-regulated by 2- to 3-fold within 1 h of hypoxia treatment in WT roots, and *rbohD* knockout was sensitive to waterlogging. Hypoxia also induced a Ca^{2+} increase in root cells, and the increase was much less pronounced in *rbohD* than in the WT. In most tissues except the elongation zone in *rbohD*, the H_2O_2 concentration had decreased after 1 h of hypoxia, but then increased significantly after 24 h. The role of ROS-Ca^{2+} hub in plant adaptive responses to salinity was studied using reciprocal grafting between salt-tolerant pumpkin and salt-sensitive cucumber plants. Salinity stress resulted in a sharp increase in H_2O_2 production, reaching a peak 3 h after salt treatment. This enhancement was accompanied by elevated expression of *RbohD* and *RbohF* and a higher NADPH oxidase activity. However, this increase was much delayed in the self-grafted plants. DPI pretreatment resulted in the loss of the salt tolerance. Inhibition of the NADPH oxidase-mediated H_2O_2 signaling in the root also abolished a rapid stomatal closure in the pumpkin-grafted plants. Taken together, our results indicate that RBOH shapes hypoxia- and salt stress-specific Ca^{2+} signatures via the modulation of apoplastic H_2O_2 production.

P 3

PLANT AUTOPHAGY IN CROSSTALK BETWEEN OXIDATIVE STRESS, REDOX SIGNALING AND ENERGY METABOLISM

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Objectives

An intricate link exists between metabolic and signaling pathways that control redox status, energy metabolism and autophagy in plants. Autophagy is an evolutionary conserved pathway that degrades oxidized, damaged and malfunctioning cellular macromolecules and organelles. Here we studied interplay between production of reactive oxygen species (ROS), induction of autophagy, activity of AMP-dependent protein kinase SnRK1 and energy status in wheat seedlings in response to abiotic stresses.

Materials and Methods

Triticum aestivum seedlings were stressed by treating with prooxidants, low positive temperature, wounding, and desiccation. Roots and leaves were tested for the levels of ROS, lipid peroxidation, cellular viability, mitochondrial potential ($\Delta\Psi_m$), nucleotides, and the activity of SnRK1 genes. Autophagy was monitored by visualizing autophagosomes and analyzing the expression of autophagic (ATG) genes.

Results

In wheat seedlings abiotic stresses induced the formation of autophagosomes and upregulated *ATG4* and *ATG8* genes. These processes were accompanied by a decrease in $\Delta\Psi_m$ and a lowering in cellular energy status. We found that a molecular marker of macroautophagy ATG8 from the ubiquitin superfamily contains W- and L-sites, which are necessary for the interactions with ligands, including ATG4. Cysteine protease ATG4 is a direct target for redox modifications. Autophagy was also induced in response to pharmacological agents that significantly increased membrane permeability or inhibited the activity of mitochondrial ETC causing ROS accumulation and energy deficit. Low energy status can activate SnRK1, which removes the block of the Target of Rapamycin (TOR) and triggers the autophagic machinery. Observed upregulated expression of SnRK1 genes in response to prooxidants suggests the involvement of this energy sensor in ROS-induced autophagy.

Conclusion

The mechanisms that control fine redox tuning of ATG4/ATG8 and the active center of SnRK may contribute to pro-survival or pro-death functions of autophagy in stressed plants.

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P 4

EFFECTS OF SALT STRESS EXPOSURE IN *ORYZA SATIVA* PLANTS: A FOCUS ON THE H_2O_2 SIGNATURE AND ANTIOXIDATIVE PROFILE

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Plants continuously cope with adverse environmental conditions, which can affect redox metabolism, leading to ROS over-production. An intricate redox network, finely modulated within cells in response to different environmental stimuli, is responsible for the generation of specific ROS signature that is pivotal for the activation of homeostatic responses against stress and for ensuring plant fitness in resistant species. The comprehension of resistance mechanisms represents an interesting research area also for their implications in food security. In this context, rice is an interesting species for its agronomic relevance and for being one of the most sensitive cereals to abiotic stresses, salinity first. In order to reach a knowledge improvement on signalling pathways triggering defence responses against salt stress, two rice varieties showing different salt sensitivity have been investigated, with particular attention to leaves and roots.

Analysis of key pathways regulating ROS production/scavenger and cellular viability/development have been performed at level of metabolic profiles, gene expressions and enzyme activities, in rice varieties after exposure to salt stresses with different intensity. Moreover, redox-dependent regulatory mechanisms, such as post-translational modifications, i.e. thiol-disulphide switch and glutathionylation, modulating enzyme activity and gene expression, have been analysed in responses to salt stress.

Data here presented indicate that plants with different capability to counteract salt stress are characterized by different basal metabolic profiles as well as by different capability to respond to the stress condition by modifying, at different levels, various players of redox metabolism. Our results contribute to describe ROS and different antioxidative pathways as a part of a complex redox network for optimizing plant responses against salinity stress.

A better knowledge of the mechanisms acting in tolerant varieties will also allow the identification of effective strategies aimed at increasing the resilience toward salt stress of rice, one of the main source of food for humans worldwide.

P 5

DEFINING H_2O_2 SIGNALING FROM MITOCHONDRIA AND CHLOROPLAST TO THE NUCLEUS IN *CHLAMYDOMONAS REINHARDTII*

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Reactive oxygen species (ROS) are mainly produced in the mitochondrial (Larosa and Remacle, 2018) and in the photosynthetic electron transport chains (Pospíšil, 2009). Historically, ROS were only considered as toxic molecules for cells, leading to oxidation of proteins, lipids and DNA.
Nowadays, the ROS-molecule H_2O_2 is increasingly being recognized as a signaling molecule due to the fact that it is relatively stable compared to the other ROS-molecules and H_2O_2 can potentially travel across membranes (Mittler, 2017). H_2O_2 signals via rapid reactions with protein cysteine sulfurs, which results in an altered protein structure and function (Pedre *et al*., 2018). Such cysteine modifications are known as S-sulfenylations (-SOH).
So far, hundreds of sulfenylated proteins have been identified in the model plant *Arabidopsis thaliana* (Waszczak *et al.,* 2014; Akter *et al.,* 2015). In this project we want to (i) identify *C. reinhardtii* crucial redox enzymes which effect the phenotype under H_2O_2-stress inducing conditions; (ii) trap and identify sulfenylated proteins involved in the redox signaling, using dimedone-based carbon nucleophiles and mass spectrometry; (iii) in vitro characterize the oxidation kinetics and the oxidation induced structural changes on one of the identified redox-sensing proteins.

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REFERENCES

1. Akter S. *et al.,* (2015) DYn-2 Based identification of *Arabidopsis* sulfenomes. *Mol. Cell Proteomics* 14:1183–1200
2. Larosa V. and Remacle C. (2018) Insight the respiratory chain and oxidative stress. *Bioscience Rep.* 38 BSR20171492
3. Mittler R. (2017) ROS are Good. *Trends Plant Sci.* 22:11-19
4. Pedre B. *et al.,* (2018) Structural snapshots of OxyR reveal the peroxidatic mechanism of H_2O_2 sensing. *Proc. Natl. Acad. Sci*. 115:E11623-E11632
5. Pospíšil P. (2009) Production of reactive oxygen species by photosystem II as a response to light and temperature stress. *Biochim Biophys Acta* 10:1151-1160
6. Waszczak C. *et al.,* (2014) Sulfenome mining in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci*. 111:11545-11550

P 6

THE ROLE OF REACTIVE ELECTROPHILE SPECIES IN THE ACCLIMATION TO HIGH LIGHT

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Singlet oxygen ($^1O_2$) is produced in the chloroplast during photosynthesis and induces transcriptional changes in the nucleus, far from its reach. The downstream molecular players in $^1O_2$ signalling, and the contribution these make to stress acclimation, remain to be clarified. Here, using a combined molecular, biochemical and physiological approach, evidence is presented for how reactive electrophile/carbonyl species (RES) are involved in the acclimation of the green alga *Chlamydomonas reinhardtii* to high light. Light stress led to an accumulation of RES, such as 2-propenal (acrolein) and 4-hydroxynonenal (HNE), which are released when $^1O_2$-derived lipid peroxides break down. Treating cells with RES stimulated similar responses to high light, including higher tolerance to $^1O_2$, similar patterns of protein carbonylation and increased levels of glutathione, an important component of RES detoxification. A RNA seq. analysis revealed clear overlaps in gene regulation between RES-treated and high light-treated cells, which included an upregulation of glutathione metabolism, carotenoid and ubiquinone biosynthesis. However, most prominent was the overlap in down-regulated genes, whereby 70% of the down-regulated genes under high light were also down-regulated by RES. Moreover, the majority of these were shared when cells were treated with Rose Bengal, as an exogenous $^1O_2$ source, confirming the role of this ROS in the RES signalling pathways under light stress. Highly represented in this gene cluster were central carbon and nitrogen-based primary metabolic pathways. Finally, a comparison to H_2O_2-mediated signalling of *C. reinhardtii* revealed that half of the genes were also differentially expressed in the same direction after treatment with HNE, the only RES that increased upon treatment of cells with H_2O_2. Therefore, HNE is also a possible pathway for H_2O_2-mediated signalling.

P 7

POST-TRANSLATIONAL REGULATION OF ORGANELLE-INDUCED STRESS RESPONSES

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Light-dependent organellar retrograde signals are vital in determining appropriate stress reactions in plants. We have identified Protein Phosphatase 2A (PP2A) as a cytosolic factor that modulates light acclimation and pathogenesis responses in *Arabidopsis thaliana*. PP2A regulatory subunit B'γ (PP2A-B'γ) is required to maintain growth and prevent premature developmental leaf senescence under favorable conditions. On a molecular level, PP2A-B'γ controls a network of proteomic and metabolic alterations elicited by organellar ROS signals. Here we have utilized genetic, proteomic and metabolomics approaches to further elucidate the function of PP2A-B'γ in cellular signalling. PP2A-B'γ physically interacts with and regulates the phosphorylation level of ACONITASE 3 (ACO3). Both knock-down *pp2a-b'γ* and phosphomimetic *aco3 pACO3:ACO3*^{S91D}*-YFP* lines display constitutively increased abundance of ALTERNATIVE OXIDASE 1A (AOX1A) and SULPHOTRANSFERSAE 12 (SOT12), key enzymes that accumulate upon activation of mitochondrial dysfunction responses. SOT12 also directly interacts with PP2A -B'γ, which mediates down-regulation of SOT12 abundance during recovery from stressful periods. Also the promoter of *PP2A-B'γ* itself is a target for transient inactivation by organellar dysfunction signals. Consequently, PP2A-B'γ does not prevent the onset of stress responses upon abrupt environmental challenges. Rather, PP2A-B'γ is essential as a post-translational regulator that prevents unnecessary stress reactions and restores growth and development upon stress relief.

P 8

ISOPRENE, MONO- AND SESQUITERPENES CONFER NO-MEDIATED IMMUNITY IN NEIGHBORING *ARABIDOPSIS* PLANTS THEREBY ENHANCING THE RESISTANCE TO MICROBIAL PATHOGENS

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Volatile organic compounds (VOCs) have many important ecological and biological functions. They are involved in repelling herbivores, attracting herbivore enemies, and function as signals in plant-microbe and plant-plant communication. By growing *Arabidopsis thaliana* in the presence of different volatile terpenes (isoprene, monoterpenes (α/β-pinene), sesquiterpenes (β-caryophyllene, (-)-thujopsene)) or VOCs naturally emitted from fungi and plants, we found that specific compounds induce plant resistance against the pathogen *Pseudomonas syringae* pvt *tomato*. Isoprene and pinenes were involved in salicylic acid (SA) mediated immunity, whereas the response to β-caryophyllene depended on jasmonic acid (JA) signaling. Moreover, the exposure of Arabidopsis plants to these volatiles triggered large changes in gene expression and in proteome-wide S-nitrosylation patterns.

We challenged Arabidopsis Col-0 and different mutant lines compromised in SA or JA signaling in closed (1) and open exposure systems with different concentrations of synthetic volatiles or biogenic VOCs emitted by the ectomycorrhizal fungus *Laccaria bicolor* (2), *Populus* x *canescens* isoprene emitting and non-emitting lines (3), and a β-caryophyllene synthase overexpressing Arabidopsis line (TPS21OE). Changes in the S-nitrosylation pattern were measured using resin-assisted capture of protein S-nitrosothiols (SNO-RAC (4)) followed by LC-MS/MS analysis.

Due to the observed VOC-induced changes in the S-nitroso-proteome, we propose that nitric oxide (NO) signaling, via posttranslational protein modification, plays an important role in mediating the induction of plant defense in the “receiver” plant. A direct scavenging (5) or an indirect action via the modulation of ROS- and NO-related signalling pathway by isoprene have been postulated as alternative mechanisms (6). Isoprene, and other terpenes, may react intracellularly with ROS and RNS radicals like H_2O_2 or NO and thus change the concentration and functionality of these molecules. Hypersensitive response (HR) is induced by changes in ROS/RNS concentrations, and the S-nitrosylation of proteins seems to be an important regulator altering susceptibility and resistance to abiotic and biotic stresses and other environmental influences (7).

1. M. Riedlmeier, A. Ghirardo, M. Wenig, C. Knappe, K. Koch, E. Georgii, S. Dey, J. E. Parker, J. P. Schnitzler, A. C. Vlot, Monoterpenes support systemic acquired resistance within and between plants. The Plant Cell 29, 1440–1459 (2017).
2. F. A. Ditengou, A. Müller, M. Rosenkranz, J. Felten, H. Lasok, M. Miloradovic van Doorn, V. Legué, K. Palme, J. P. Schnitzler, A. Polle, Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprograms root architecture. Nat. comm. 6, 6279 (2015).
3. K. Behnke, B. Ehlting, M. Teuber, M. Bauerfeind, S. Louis, R. Hänsch, A. Polle, J. Bohlmann, J. P. Schnitzler, Transgenic, non-isoprene emitting poplars don’t like it hot. The Plant Journal 51, 485–499 (2007)
4. M. T. Forrester, J. W. Thompson, M. W. Foster, L. Nogueira, M. A. Moseley, and J. S. Stamler, Proteomic analysis of S-nitrosylation and denitrosylation by resin-assisted capture. Nat Biotechnol. 27(6), 557–559 (2009)
5. V. Velikova, T. Tsonev, P. Pinelli, G. A. Alessio, F. Loreto, Localized ozone fumigation system for studying ozone effects on photosynthesis, respiration, electron transport rate and isoprene emission in field-grown Mediterranean oak species. Tree Physiol. 25(12), 1523-32 (2005b)
6. E. Vanzo, J. Merl-Pham, V. Velikova, A. Ghirardo, C. Lindermayr, S. M. Hauck, J. Bernhardt, K.Riedel, J. Durner, J. P. Schnitzler, Modulation of protein S-nitrosylation by isoprene emission in poplar. Plant Phys. 170 (4), 1945-1961 (2016)
7. J. Astier, A. Kulik, E. Koen, A. Besson-Bard, S. Bourque, S. Jeandroz, O. Lamotte, D. Wendehenne, Protein S-nitrosylation: what's going on in plants? Free Radic Biol Med. 1;53(5), 1101-10 (2012)

P 9

LINKING THE CHLOROPLAST NTRC THIOREDOXIN SYSTEM TO MITOCHONDRIAL AOX ACTIVITY

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Increased activity of mitochondrial alternative oxidases (AOXs) affects chloroplast ROS processing and thiol redox states of chloroplast enzymes [1]. Additionally, in the AOX-overexpressing Arabidopsis mutant *rcd1* the chloroplast plastoquinone pool is over-reduced in the darkness or after treatment with the AOX inhibitor SHAM. The molecular mechanisms of this trans-organellar interaction remain unclear. We first addressed whether the chloroplast NADPH-dependent thioredoxin reductase C (NTRC) was involved in this interaction. This enzyme is implicated in chloroplast thiol redox control and in regulation of chloroplast cyclic electron flow [2]. Biochemical and spectroscopic studies indicated that the reduction of plastoquinone pool observed in *rcd1* was absent from the *rcd1 ntrc* double mutant. This double mutant also had decreased tolerance of Photosystem II to chloroplastic ROS, compared to *rcd1*. These observations suggest that in the situation of increased AOX activity the NTRC system alters chloroplast electron flows and chloroplast ROS processing. Next, chloroplast electron transfer was assessed by PAM and OJIP imaging of leaf discs pre-treated with methyl viologen. This chemical catalyses Mehler reaction, which is the transfer of electrons from Photosystem I to molecular oxygen. The short-term effect of methyl viologen on the chloroplast linear electron flow was similar in the wild-type and *rcd1* plants in aerobic conditions. However, under hypoxia the oxidative effect of methyl viologen was absent from *rcd1*, although still observed in the wild type. This phenomenon was likely associated with suppressed Mehler reaction and independent from PTOX. The possibility of increased AOX activity affecting oxygen availability at the electron-acceptor side of Photosystem I is discussed.

1. Shapiguzov, A., J.P. Vainonen et al. Arabidopsis RCD1 coordinates chloroplast and mitochondrial functions through interaction with ANAC transcription factors. eLife, *2019*. pii: e43284

2. Nikkanen, L. et al. Regulation of cyclic electron flow by chloroplast NADPH-dependent thioredoxin system. Plant Direct, *2018*. 2 (11): e00093

P 10

DEVELOPMENTAL EXPRESSION AND SUBCELLULAR LOCALISATION OF IRONIC SUPEROXIDE DISMUTASE 1 IN *ARABIDOPSIS*

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Superoxide dismutases (SODs) are key antioxidant enzymes responsible for the deactivation of superoxide radical by catalysing its dismutation to hydrogen peroxide and oxygen. It is known that SODs determine plant abiotic stress tolerance, but the knowledge about their *in vivo* develpomental expression and *in vivo* subcellular localisation is still elusive. Here we address the organ- and tissue- specific developmental expression patterns, as well as subcellular localisation of ironic SOD FSD1 using modern fluorescence microscopy methods in Arabidopsis. Therefore, we stably expressed FSD1 fused to GFP from both N- and C- termini in *fsd1* mutant background under its own native promotor. In roots, both C- and N- terminal GFP fusions of FSD1 showed high fluorescence intensity in root initials, epidermis, columella and lateral root cap. We also observed an intense signal in aboveground organs, namely cotyledons, hypocotyls and petioles. Notably, FSD1 was specifically accumulated in lateral root primordia corroborating the fact that *fsd1* mutants exhibit reduced number of lateral roots. At the subcellular level, the FSD1-GFP construct localized to plastids, nuclei and cytoplasm. The fusion of GFP to FSD1 at the N terminus blocked the plastid targeting of FSD1 leading to the redistribution of its plastidial pool to nucleus and cytoplasm. While *fsd1* mutants expressing C-terminal fusion of FSD1 (FSD1-GFP) exhibited wild type-like response to oxidative stress induced by paraquat, the N-terminal fusion (GFP-FSD1) caused only partial complementation of *fsd1* mutants. These results show that in addition to plastidial FSD1, nuclear and cytoplasmic FSD1 pools are also required for plant oxidative stress tolerance.

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P 11

DEFINING THE INTER-ORGANELLAR REDOX COMMUNICATION IN *CHLAMYDOMONAS REINHARDTII*

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Plants rely on signal transduction events to respond, adapt and survive under various environmental stress conditions. One of these signals are reduction-oxidation (redox) signals. How these redox signals are perceived, triggered, and transduced is still not fully understood. To define redox communication, we use the green microalgae model system *Chlamydomonas reinhardtii.* Firstly, we follow how the levels of the redox metabolite H_2O_2 changes in organellar cross-talk experiments using the HyPer redox biosensor (Bilan and Belousov, 2016). As a rapid first redox response, H_2O_2 reacts with cysteine thiols on proteins, forming sulfenylated cysteines. Therefore, secondly, we trap and identify these cysteine-modified proteins in the organelles where we observe an increase of the H_2O_2 levels using the genetic YAP1C probe (Waszczak *et al.*, 2014; Smet *et al.*, 2019). Thirdly, we aim to study the oxidation kinetics and the oxidation induced structural changes on one of the redox-sensing proteins from a curated list of organellar proteins. With this approach, we will get new perspectives on organellar H_2O_2 communication and on the pathways that might be engineered to help the photosynthetic cells to cope with H_2O_2-stress inducing environmental conditions.

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References:

Bilan, D.S., and Belousov, V. V. (2016) HyPer Family Probes: State of the Art. *Antioxid Redox Signal* 24: 731–751

Smet, B. De, Willems, P., Fernandez-fernandez, A.D., and Alseekh, S. (2019) In vivo detection of protein cysteine sulfenylation in plastids. *Plant J* 97: 765–778.

Waszczak, C., Akter, S., Eeckhout, D., Persiau, G., Wahni, K., Bodra, N., *et al.* (2014) Sulfenome mining in Arabidopsis thaliana. *Proc Natl Acad Sci* 111: 11545–11550

P 12

DEVELOPMENTAL MONITORING OF GLUTATHIONE REDUCTASE ACTIVITY, EXPRESSION, CELL LOCALIZATION AND MOLECULAR VARIABILITY REVEALS ITS RELEVANCE IN THE MAINTENANCE OF REDOX HOMEOSTASIS IN MALE GAMETOPHYTE OF THE OLIVE TREE (*OLEA EUROPAEA* L.)

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Glutathione is a tripeptide of low molecular weight present in most plant cells, determining correct development and physiology throughout its antioxidant character, concomitant with additional roles in Sulphur assimilation, heavy metal detoxification, gene expression, signaling etc. Glutathione can be present in both its oxidized (GSSG) and reduced (GSH) forms, with natural predominance of the later under non-stressing conditions. Glutathione reductase (GR; E.C. 1.8.1.7) represents the major enzyme activity converting GSSG into GSH by using NADPH. The key importance of this enzyme in maintaining redox homeostasis of plant tissues under different conditions has been widely reported. However, our knowledge on the presence, activity, localization and variability of this enzyme in the plant reproductive tissues is rather limited. In the present work we have determined the presence of numerous GR transcripts in the reproductive transcriptome of the olive tree (ReprOlive), which have been deeply analysed by using bioinformatics tools to determine phylogenetic relationships with other plant GR sequences, predict their physic-chemical properties, their potential post-translational modification, putative cell localization, and predicted 3-D structure.

In addition to predictive tools, a broad panel of biochemical analyses has been performed to assess and quantify overall GR activity in the developing anther, the mature olive pollen and the growing pollen tube *in vitro* at different developmental stages. Presence of specific variants of GR has also been assessed by means of *in gel* activity assays. The activity of the enzyme was differentially inhibited in the presence of the GR inhibitors L-carmustinine, 2-AAPA acetylamine and BSO in the reproductive e tissues. Heterologous antibodies from *A. thaliana* were used to identify and quantify the different forms of the protein present in pollen extracts. Moreover, immunocytochemical analyses using the same set of antibodies confirmed cell localization of the enzyme over the bioinformatic prediction. Finally, the expression of the GR forms detected in the reproductive tissues was assessed by q-PCR. The mRNAs corresponding to the enzyme displayed conspicuous changes along development, and in the different tissues analysed, thus providing evidence of the importance of the enzyme in the reproductive process.

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P 13

DISTINCT IMPACTS OF CYTOSOLIC AND CHLOROPLASTIC ASCORBATE PEROXIDASES ON CELL DEATH IN CATALASE-DEFICIENT PLANTS

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H_2O_2 plays a dual role being cytotoxic molecule and having signaling roles in plant stress response. Arabidopsis catalase 2 (CAT2), one of three CAT isoforms, has a predominant role in scavenging H_2O_2 in leaf peroxisomes, which are one of the most significant sources for H_2O_2 production. *cat2* mutant shows a severe cell death phenotype under high light stress but, interestingly, the cell death is known to be alleviated by the lack of cytosolic ascorbate peroxidase (APX1). However, the molecular mechanism of this paradoxical phenotype is not fully understood. It is also unclear whether the cell death alleviation is also caused by the lack of other APXs with different sub-cellular localization, such as chloroplastic isoforms (stromal sAPX and thylakooid membrane tAPX). We herein compared the impacts of cytosolic and chloroplastic APXs on the *cat2* cell death.
Under high light stress, *cat2* showed a severe cell death phenotype, which was accompanied by accumulation of oxidized glutathione (GSSG) and by decrease in total ascorbate pool. Consistent with previous reports, the cell death in *cat2* was alleviated by the lack of APX1. In addition, the GSSG accumulation and ascorbate depletion were also mitigated in *apx1 cat2*. These findings suggest that cytosolic APX1 facilitates glutathione oxidation and ascorbate degradation (or consumption) to trigger the cell death in the absence of CAT2. In contrast, the lack of chloroplast APXs did not alleviate the cell death, GSSG accumulation and ascorbate depletion in *cat2*. Rather, *sapx tapx cat2* triple mutant was tended to be more sensitive to high light compared with *cat2* single mutant. Thus, the impacts of chloroplast APXs on *cat2* are completely different from those of cytosolic one. Further analyses, including measurements of stress-related hormones (salicylic acid and jasmonic acid), using *apx1 sapx tapx cat2* quadruple mutants, are ongoing.

P 14

P700 OXIDATION SUPPRESSES THE PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS) IN PHOTOSYSTEM I OF THYLAKOID MEMBRANES: ROBUST MECHANISMS TO ALLEVIATE OXIDATIVE DAMAGES IN PHOTOSYNTHESIS ORGANISMS

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MAIN OBJECTIVE: Lower efficiency of light use in suppressed photosynthesis under environmental stress is considered to induce production of reactive oxygen species (ROS) in photosystem (PS) I of thylakoid membranes. We tried to elucidate the regulation mechanisms of ROS production, otherwise the accumulated ROS attacks and causes death to photosynthesis organisms.

MATERIALS & METHODS: Green-lineage photosynthesis organisms: cyanobacteria, green algae, liverworts, ferns, gymnosperms, angiosperms, and red-lineage ones: red algae, brown algae, diatoms were used. Chlorophyll fluorescence and redox reaction of reaction center chlorophyll (P700) in PSI were simultaneously analyzed by DUAL-PAM (Walz, Germany).

RESULTS: Intact leaves of angiosperms were repetitively illuminated by saturated-pulse light (rSP-illumination: 20,000 mmol photons m^{-2} s^{-1}, 300 ms duration, every 10 sec) to accumulate electrons in PSI in the dark. As rSP-illumination time increased, PSI was oxidatively inactivated, as judged by decrease in P700 content. Contrarily, PSII did not suffer from any photoinhibition. Furthermore, rSP-illumination did not inactivate PSI under continuous actinic-light illumination, where P700 was oxidized. The other green-lineage photosynthesis organisms, except for angiosperms, do not suffer from any PSI inactivation by rSP-illumination in the dark. In these organisms P700 was oxidized during saturated-pulse by flavodiiron protein (FLV), which catalyzes NADPH-dependent O_2-reduction to water at PSI. FLV-deficient mutant of liverwort, *Marchantia polymorpha*, did not show any P700 oxidation during saturated-pulse, and suffered from PSI inactivation. Interestingly, the red-lineage photosynthesis organisms did not suffer from any PSI inactivation by rSP-illumination in the dark. Surprisingly, these organisms do not have any FLV genes, and can oxidize P700, but the underlying oxidation mechanisms remain unknown.

DISCUSSION: From these findings, we hypothesize that P700 oxidation decreases the excited P700, the electron source for O_2-reduction, to suppress ROS production in PSI. Generally, photosynthesis organisms oxidize P700 under high light and low CO_2. These are robust responses to suppress oxidative damages.

P 15

REGULATION OF HISTONE DEACETYLASE ACTIVITY IN *ARABIDOPSIS* BY REDOX-SPECIES

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Histone acetylation is one major mechanism of epigenetic transcription control. The acetylation mark of chromatin is removed by the action of histone deacetylases (HDACs) and it could be shown that this enzymatic process is modulated by redox compounds like nitric oxide (NO) in several mammalian HDACs, e. g. human HDA2. *Arabidopsis* crude extracts or protoplasts displayed a diminished total HDAC activity when treated with a physiological NO-donor, as well as H3K9ac marks were increased in the treated samples, suggesting the presence of NO-regulated plant HDACs. However, the redox-regulated HDACs in *Arabidopsis* are still not identified. A conducted BLAST search using the amino acid sequence of redox-sensitive human HDAC2 identified *Arabidopsis* HDA6 and HDA19 as highly homologous plant HDACs that share the NO-sensitive cysteines with HDAC2. Moreover, we could show that *Arabidopsis* HDA5 emerges as a promising candidate for redox-regulated HDAC. An attempt to purify NO-sensitive HDACs in their active form from *Arabidopsis* seedlings and cell culture identified HDA5 as the only HDAC present in all redox-sensitive chromatography fractions. Interestingly, HDAC activity from these fractions was increased after treatment with the NO-donor *S*-nitroso-glutathione. This effect could be confirmed by experiments with recombinantly produced HDA5. Furthermore, treatment with oxidized glutathione also increased the activity, while hydrogen peroxide diminished it. These results indicate a novel mechanism for differentially regulation of HDAC activity by redox-species in plants.

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SIGNAL INTERACTION BETWEEN NITRIC OXIDE (NO) AND RHO GTPASES DURING ROOT GROWTH

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Nitric oxide (NO) in cooperation with auxin regulates primary root or root hair elongation and lateral root formation. Some of these physiological processes take place with the participation of plant-specific regulator Rho of Plants (ROP) proteins which are known to be involved in auxin transport and signaling. Although, NO and ROPs as regulators share common targets (auxin transport/signaling) and have roles in similar physiological process (root growth), their crosstalk has not been proven so far. Our study aims therefore to examine a suspected NO-ROP signal interplay in plants. We applied S-nitrosoglutathione (GSNO) or S-nitrosopenicillamine (SNAP) as exogenous NO donors (for 72 hours) and evaluated the NO sensing by quantifying primary root shortening in the presence of NO.
Compared to the wild-type (*Col-0*), *rop2-1* and *rop2-2* roots showed significant NO insensitivity, while *rop6* responded to the presence of NO donors similarly to *Col-0* (GSNO/SNAP-induced root meristem shortening). In agreement with this, neither the rate nor the pattern of ROP6 *in situ* expression was affected by NO supplementation. Moreover, complementation of ROP2 mutation improved NO sensing. The *in situ* expression of ROP2, as well as the PIN-dependent auxin transport and auxin maximum, decreased in the presence of NO in the root tip. Based on the results, we can strongly suspect that exogenous NO influences ROP2 action thus inhibiting polar auxin transport and consequently the generation of auxin maximum that leads to root meristem shortening.

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P 17

CHARACTERIZATION OF A NOVEL OXIDATIVE STRESS-RESPONSIVE METHYLTRANSFERASE IN *ARABIDOPSIS THALIANA*

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Transcriptional reprogramming induced by reactive oxygen species (ROS) perturbation can lead to the adjustment of development and growth processes, defense responses or programmed cell death events. The functional analysis of proteins that are encoded by genes that rapidly respond to increased ROS levels can give insights into the signalling or metabolic events of plant stress response.

Methylation reactions are vital in determining various physiological processes in living organisms, such as primary and secondary metabolism, epigenetic modification and post-translational control of protein function. Here, the function of a novel oxidative stress-responsive methyltransferase called EAL4 (EMBRYONIC ABUNDANT PROTEIN-LIKE-RELATED 4) in *Arabidopsis thaliana* was investigated. The transcript level of EAL4 gene is strongly induced by oxidative stress. Significantly, EAL4 overexpression confers resistance to toxic concentrations of cantharidin, a potent inhibitor of protein serine/threonine phosphatases. Through an IP-MS analysis, we identified the EAL4 interactome, indicating an enriched interaction with nitrilases and various enzymes involved in acetyl-coA metabolism. Overall, these results provide insights into the potential link between oxidative stress and methylation.

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NPR1 REGULATED REACTIVE OXYGEN SPECIES SIGNALLING IN SOYBEAN UNDER STRESS

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Non-expressor of pathogenesis-related gene (NPR1) is a key regulator of the SA-dependent defence response and systemic acquired resistance (SAR) in plants. Although NPR1 is a well known important regulator of salicylic acid to pathogen defence, researching on NPR1 in plants under salt stress and interaction with reactive oxygen species (ROS) is limited and have not yet been well established. Given that, salicylic acid involves in the regulation of salt tolerance mechanisms by the activation of plant defence responses, such as, converse of oligomer NPR1 to monomer, modulate redox state, increase antioxidant protection, maintain ion balance, enhance H^+-ATPase activity and improve photosynthethic capacity, NPR1 might play role in mediating defence response, redox signaling, maintaining hormone level, inducing antioxidant defence system, preventing programmed cell death in plants. To understand the functions of NPR1 will be guide for analyse in plant salt tolerance. This is the first report on comparative analysis of NPR1 under salt and PEG induced osmotic stress.

With this aim, soybean (*Glycine max* L.) (SA88) plants were grown with Hoagland solution for two weeks. Seedlings were treated with 200 mM NaCl, 10% PEG 6000 and 200 mM NaCl + 10% PEG 6000. Relative growth rate (RGR), relative water content (RWC), relative electrolyte leakage (REL), abscisic acid (ABA) and salicylic acid (SA) level, lipid peroxidation, reactive oxygen species content and the differences in *Gm-NPR1* and thioredoxin h5 (*TRXh5*) gene expressions were determined comparatively.

These findings provide new insight into the interaction of salt or PEG induced ROS and NPR1 signalling in soybean leaves to increase salt tolerance.

P 19

COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA*): SYSTEMS BIOLOGY OF THE ALLERGENIC POLLEN UPON ELEVATED NO_2 CONCENTRATIONS

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The pollen of ragweed (*Ambrosia artemisiifolia*) is one of the strongest allergy triggers known and can cause allergic reactions such as asthma very quickly. Global warming and changes in the environment (e.g. air pollution, heavy metal ions, etc.) will result in an earlier and longer pollen season, enhanced pollen production and an increase in pollen allergenicity with a negative effect on atopic patients. The objective of this study was to investigate the effects of NO_2, a major air pollutant, under controlled conditions, on the allergenicity of ragweed pollen. Ragweed plants were exposed to 80 ppb NO_2 over a whole vegetation period. Elevated NO_2 caused significant decrease on the seed production, whereas the pollen production was increased. Comparative transcriptome, proteome and immunoblot analysis of ragweed pollen revealed that the allergen transcripts were up-regulated at the transcriptional and protein level meanwhile the pollen allerginicity as tested by immunoreaction, was also increased. On the other hand, the major allergen Amb a 1 was nitrosylated under low and elevated NO_2-conditions, indicating that nitrosylation, similar to nitration, may influence the allergenic potential of pollen. This NO_2 influence on ragweed pollen may induce negative effects on atopic patients.

P 20

ROLE OF ARGONAUTE PROTEINS IN POST-TRANSCRIPTIONAL REGULATION OF EXPRESSION OF GENES INVOLVED IN ROS METABOLISM DURING LIGHT-DEPENDENT GERMINATION OF *ARABIDOPSIS* SEEDS

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Seed dormancy and germination are complex processes controlled by environmental (i.e. light) and endogenous factors (i.e. reactive oxygen species, ROS), requiring several changes in cells including these operating at post-transcriptional level. The specific genes coding proteins of the great importance in modulation of the cross-talk between ROS and light in seed related events are not yet characterized. It was shown that regulatory proteins coded by *ARGONAUTE (AGO)* genes (from *AGO1* to *AGO10* in *Arabidopsis*) play important and diverse roles in regulation of various aspects of plant development. Some of these proteins (i.e. AGO1) are elements of the RNA-induced silencing complex (RISC), which use small RNAs to select targets for silencing. Up to date, it is not known whether AGO1 is involved in regulation of light/ROS-dependent processes occurring in seeds. Therefore, the aim of this study was to elucidate the possible crosstalk between AGO-related regulatory mechanism and ROS metabolism in light-dependent germination of *Arabidopsis* seeds. The *Arabidopsis* dormant and after-ripened seeds were used in biological assays performed in different light conditions, on water or in the presence of DPI (diphenylene iodonium; an inhibitor of NADPH oxidases activity). To characterize the effect of light on the relative level of transcripts of genes of *AGO1* and related to ROS metabolism (*RBOHB, RBOHD*) in germinating *Arabidopsis* seeds, the qRT-PCR analysis was performed. In addition, *in situ* ROS localization in seeds germinating in different light conditions was performed using a NBT (nitroblue tetrazolium) staining, while an abundance of AGO1 protein was identified by Western blot. The obtained novel results indicated that AGO1 may be involved in the regulation of light-dependent germination *via* modulation of ROS metabolism.

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NITRIC OXIDE (NO) INVOLVEMENT IN THE REGULATION OF THE BIFUNCTIONAL *ENO2/MBP1* LOCUS

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Glycolytic enzymes are highly conserved in prokaryotic and eukaryotic organisms, and enolase (ENO2) is among the most abundant cytosolic proteins. ENO2 is essential for the growth and development of plants (Eremina et al., 2015). Defects in ENO2 function yield plants with severe developmental defects and constitutively reduced growth (Eremina et al., 2015). ENO2 can be regulated by different post-translational modifications (PTMs) (Pearlman et al., 2011). In particular ENO2 S-nitrosylation seems to play a major role in the activity of this enzyme (Zhang et al., 2017). However how this PTM, which is directed by nitric oxide (NO), affects ENO2 activity and possibly also redox stability in plants is still to be deciphered. Using different genetic and pharmaceutical approaches, we show that clear changes in the enzymatic activity of ENO2 are correlated with alterations in NO levels in the plant. In addition to an enolase the *ENO2* locus also encodes the transcription factor AtMBP-1, which feedback regulates ENO2 abundance. Studies of how NO regulates AtMBP1 to maintain ENO2 homeostasis are on their way to a better understanding of this crucial enzyme in plants.

Eremina et al., 2015. The Plant Journal, 81, 895–906.
Pearlman et al., 2011. Cell, 147 (4), 934-946.
Zhang et al., 2017. Plant, Cell and Environment, 40, 1834–1848.

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MOLECULAR STRUCTURE AND VARIABILITY OF GLUTATHIONE *S*-TRANSFERASES IN THE OLIVE REPRODUCTIVE TISSUES

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Glutathione *S*-transferases (GST) (EC 2.5.1.18) represent a superfamily of enzymes catalyzing the reaction between the antioxidant tripeptide glutathione in its reduced form (GSH) and electrophilic centers present on a wide variety of substrates in order to make the compounds more water-soluble and therefore participating in detoxification of different compounds (i.e. xenobiotics and toxins). The sequence and the structure of several plant GSTs have been resolved, however the information regarding these enzymes in plant reproductive tissues is scarce and fragmentary.

The present work describes the sequence and the molecular structure of a panel of GSTs, retrieved from the ReprOlive database of transcripts expressed in olive reproductive tissues (http://reprolive.eez.csic.es/olivodb/). Nineteen and twenty transcripts were selected from olive pollen and olive seed transcriptomes, respectively. These sequences were subjected to a broad bioinformatic analysis, including the prediction of physical and chemical properties (Mw, IP…), their classification into groups, a predictive analysis of post-translational modifications, and the determination of the phylogenetic relationships among them and other relevant GST forms. Moreover, 2-D and 3-D predictive models were developed for several of these forms. Predictive properties are currently been assessed by means of biochemical assays

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TARGETING THE OXIDATIVE STRESS RELATED GENES IN RESPONSE TO *PHYTOPHTHORA CAPSICI* IN CHAYOTE OR MIRLITON SQUASH (*SEQUIUM EDULE* JACK SW.)

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Chayote (*Sequium edule* Jack Sw.) has economic and social importance in the state of Veracruz (Mexico), considering the main producer nationwide. Fungal diseases greatly affect the production and marketing of this crop.

In the present work, plants and fruits of *Sechium edule* were inoculated with *Phytophthora capsici* in order to assess the changes in the differential expression of oxidative stress-related enzymes in relation to *P. capsici* disease resistance.

Quantitative analysis of genes and proteins expression of enzymes involved in regulating reactive oxygen species (ROS) homeostasis system (SOD, Catalase and glutathione cycle-related genes) were determined at six post inoculation stages (2, 4, 8, 14, 24 and 48 hpi). Particularly, the amount of reduced glutathione (GSH), as well as the specific activities as glutathione reductase (GR) and glutathione-S-transferase (GST), and the total proteins oxidation were measured by immunoblotting, qRT-PCR, and enzymatic activities assays using commercial kits. The results showed pronounced quantitative difference in the glutathione cycle-related genes/proteins during the course of the *P capsici* infection, with particular differences between vegetative and fruit tissues, highly patent at 2 hpi and between 14 and 24 hpi.

These results indicate that *P. capsici* infection induced a protective and signaling response in the plant, specifically in the expression and activity of antioxidant enzymes, which mediate the tolerance of this variety of chayote to this particular strain of P. capsici. Furthermore, the results provide valuable information that could represent a useful tool for the improvement of the sustainable protection of the crop with the implementation of improvement genetic programs for *S. edule*.

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NITRIC OXIDE SUPPLEMENTATION AVOIDS SALT STRESS -INHIBITED PHOTOSYNTHETIC PERFORMANCE THROUGH INCREASED NITROGEN AND SULFUR ASSIMILATION AND ANTIOXIDANT DEFENSE SYSTEM IN MUSTARD

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Salt stress is one of the major constraints in agriculture which affects plant growth and productivity. The high concentration of salt in soil causes ionic imbalance leading to osmotic stress and subsequently oxidative damage to lipids, protein and nucleic acid in the plant cell through increased reactive oxygen species (ROS) production. Plants induce certain resistance mechanisms to avoid oxidative damage caused by salinity. Nitric oxide (NO) is recently recognized plant hormone that acts as a biological messenger in cells and participates in many physiological processes to avert adverse effects of salt stress in plants. In the present reported research, application of 100 µM NO as sodium nitroprusside (SNP; NO donor) was studied individually (100 mg N /kg soil or 100 mg S /kg soil) or in combination with split form of (50N+50N+ 50S+50S) nitrogen (N) and sulfur (S) alleviated effects of 100 mM NaCl stress in mustard (*Brassica juncea* L.). Plants receiving NO together with N and S exhibited lower superoxide ion accumulation under salt stress than the plants receiving NO and N, S alone. These plants (receiving NO + N+ S) exhibited increased N and S assimilation by increasing the activity of rate limiting enzymes ATP-sulfurylase (ATP-S), and nitrate reductase (NR), and enzymes of antioxidant defense system; super oxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). These NO induced response led to higher accumulation of cysteine (Cys), reduced glutathione (GSH) and proline with lesser oxidative stress and stimulated photosynthetic performance of plants. The study suggests that salt stress effects on photosynthetic performance of plants are mitigated more efficiently when NO was applied in combination and split form of N and S and the photosynthetic activity was promoted under salt stress through increased N and S assimilation and antioxidant system.

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SINGLET OXYGEN SIGNALLING PATHWAYS DURING DE-ETIOLATION IN *ARABIDOPSIS*

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Seedlings undergo de-etiolation when transferred from darkness into the light. This process involves development of the photosynthetic apparatus and synthesis of chlorophyll. The accumulation of chlorophyll and porphyrins, such as protochlorophyllide (Pchlide), must be tightly regulated during this time, as excitation by light can produce singlet oxygen ($^1O_2$), which at high levels leads to seedling damage or death. We have previously shown in *Arabidopsis thaliana* that excess Pchlide accumulation in dark-grown seedlings results in a $^1O_2$ burst when transferred to white light and inhibition of genes required for chlorophyll biosynthesis and development of the photosynthetic apparatus (Page *et al.* 2017).

Here we describe an EMS mutagenesis screen used to identify mutants in this $^1O_2$ signalling pathway. Seedlings grown under far-red light conditions accumulate surplus Pchlide, which upon exposure to light generates a $^1O_2$ burst, causing downregulation of the chloroplast biosynthesis gene *HEMA1*. Transgenic seedlings were produced that contained the *HEMA1* promoter linked to the *BAR* gene, which encodes for phosphinothricin (PPT) resistance. Following a far-red screen, seedlings that retained *HEMA1* expression survived, and were termed *safe after far-red* (*saf*). One of these mutants, *saf7* was identified as a *toc132* mutant using a MutMap genome sequencing approach (Abe *et al.* 2012). This was confirmed by demonstrating that *saf7* had a truncated TRANSLOCON AT THE OUTER ENVELOPE MEMBRANE OF CHLOROPLAST132 (TOC132) protein. Both *saf7* and *toc132-2* show maintenance of greening and expression of chlorophyll biosynthesis and photosynthesis genes including *HEMA1,* and *LHCB2.1* following far-red pre-treatments. Crucially, they both show similar levels of Pchlide accumulation to WT suggesting that they lack the ability to respond to elevated $^1O_2$ during de-etiolation. The nature of the role of TOC132 in $^1O_2$ signalling is currently being elucidated.

Page et al (2017) New Phytologist, 213, 1168-1180; Abe et al (2012) Nature Biotechnology 30, 174–178

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NADPH OXIDASE (*RBOH*) MEDIATED N, P, K DEFICIENCY SIGNALLING IN GLYCOPHYTE *ARABIDOPSIS THALIANA* AND HALOPHYTE *SCHRENKIELLA PARVULA*

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Aim of this work was to elucidate involvement of NADPH oxidase (*RBOH*) mediated ROS signalling in perception of N, P, K deficiency and its involvement in the nutrient acquisition response. We also aimed to see if there are differences in nutrient deficiency response of glcyophytic and halophytic plants. For this purpose, model glcyophte *Arabidopsis thaliana* and model halophyte *Schrenkiella parvula* (*Eutrema parvulum*) was used. *A. thaliana* and *S. parvula* plants were grown hydroponically with ½ Hoagland nutrient solution for 4 and 6 weeks, respectively and then they were transferred to deionized water (no nutrient) and N, P or K deficient Hoagland solution. Plants were harvested at 0, 30, 60 and 120 mins after transfer. NADPH oxidase activity was measured in this time course experiment. NADPH oxidase acitivity peaked at 30 mins after being transferred to nutrient deficient medium in both species but it was more prominent in *S. parvula* (91% increase), when compared to *A. thaliana* (45% increase). NADPH oxidase activity returned to control levels at 120 mins. Expressions of 10 RBOH genes (RBOHA-J) was also measured in both species to understand which RBOH genes are responsible for this burst in NADPH oxidase activity. Moreover, nutrient deficiency + diphenyleneiodonium (DPI) treatments will be conducted for determining changes in nutrient acquisition.

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EFFECTS OF DIFFERENT SALTS (NaCl, $NaNO_3$, Na_2SO_4) ON KINETICS OF ANTIOXIDANT ENZYMES OF GLYCOPHYTE *ARABIDOPSIS THALIANA* AND HALOPHYTE *SCHRENKIELLA PARVULA IN VITRO*

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Accumulation of various ions at high concentrations in plants cells can lead to inhibition of enzyme activity by interfering ionic and hydrophobic interactions of proteins leading impartment of metabolism. To prevent this, plants developed various mechanisms and glycophtes and halophytes differ in their ability to exclude ions from cytosol and compartmentalize them into apoplast or vacuole. It is well known that antioxidant defense capacity is a vital component of salt stress tolerance. However, effects of different ions on inhibition of antioxidant enzyme activity is not known. Therefore, the aim of this study was to test if antioxidant enzymes of glycophyte and halophyte plants differ in their resistance to inhibition by different ions (NaCl, $NaNO_3$, Na_2SO_4) *in vitro*. For this purpose, glycophytic model *Arabidopsis thaliana* and extreme halophytic model *Schrenkiella parvula* (*Eutrema parvulum*) was utilized and activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) was measured with reaction mixtures containing 50, 100, 250 or 500 mM NaCl, $NaNO_3$ or Na_2SO_4. Activities of all enzymes were inhibited gradually in both species by increasing salt concentrations expect for 50 mM $NaNO_3$, which induced POX activity by 11% in Arabidopsis and 26% in *S. parvula*. GR activity of *S. parvula* was more resistant to NaCl when compared to Arabidopsis, 100 mM NaCl decreased GR activity to 60% of control in Arabidopsis while 90% of activity was maintained in *S. parvula*. NaCl had more negative impact on GR enzyme activity when compared to $NaNO_3$. No differences were observed for CAT activity between *A.* thaliana and *S. parvula*. Overall, this work provides evidence related to effects of different ions such as Na^+, Cl^-, SO_4^{-2} and NO_3^- on antioxidant enzyme activity of glycophytes and halophytes.

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PLANT SENESCENCE, ANTIOXIDANT ACTIVITY AND DISEASE RESISTANCE

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It has been known since a long time that senescence or juvenility of plant tissues has strong effect on their reactions to pathogen attacks. Generally necrotrophic pathogens prefer senescent, while biotrophic pathogens prefer juvenile tissues. Furthermore, during pathogenic attack reactive oxygen species are formed, that can be controlled by antioxidants. On the other hand, during senescence, the antioxidant capacity of plant generally decreases. The main objectives of this study are the relations between plant senescence, antioxidant capacity and disease resistance.

Experiments were carried out with *Nicotiana tabacum* L. cv. Xanthi-nc, its salicylic acid deficient NahG mutant, a paraquat-sensitive cv. Samsun (PS) and its paraquat tolerant (PT) tobacco mutants. We used wild-type Columbia ecotype of *Arabidopsis thaliana* (L.) Heynh, its double mutant nrp1-1 nrp2-1 and an overexpressing nrp1ox lines as well. Near isogenic barley lines cv. Ingrid with or without various resistance genes to powdery mildew were also applied. As pathogens *Tobacco mosaic* virus (TMV), *Botrytis cinerea* and *Sclerotinia sclerotiorum* were used. The antioxidant enzymes were spectrophotometrically analysed. Expressions of selected genes were determined by qPCR.

NahG and PS tobaccos with faster senescence showed higher sensitivity not only to H_2O_2 and NO (SNP) treatments, but also to necrosis inducing pathogens than their respective control Xanthi and PT tobaccos. In addition, NahG and PS tobaccos had lower antioxidant activities than Xanthi and PT tobaccos, respectively. Furthermore, *Arabidopsis thaliana* double mutant nrp1-1 nrp2-1 showed significantly faster senescence, elevated expression of senescence associated SAG13 gene and lower ascorbate peroxidase and glutathione S-transferase enzyme activities, as well as higher susceptibility to the necrotrophic pathogen *Sclerotinia sclerotiorum*. Similarly, *mlo* mutant of barley with faster senescence had less tolerance to H_2O_2 treatment and to necrosis inducing pathogens.

The correlations between plant senescence, antioxidant capacity and disease resistance with further examples will be discussed.

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ARABIDOPSIS UCP1 REGULATES MITOCHONDRIA-NUCLEUS RETROGRADE SIGNALLING THROUGH THE CYS-PRT6 N-DEGRON PATHWAY

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Plant MITOCHONDRIAL UNCOUPLING PROTEIN 1 (UCP1) has been studied for a long time for its function in the uncoupling of mitochondrial respiration. Recently it has been proposed that UCP1 is also part of the malate-aspartate shuttle that exchanges aspartate/glutamate across mitochondrial inner membrane. Overexpression of UCP1 in plants triggers mitochondrial biogenesis, up-regulates a hypoxia transcriptome, and enhances plant tolerance to a broad range of abiotic and biotic stresses. Here we show that Arabidopsis thaliana (At)UCP1 regulates the shift between oxidative and non-oxidative metabolism in response to stresses via the oxygen and nitric oxide (NO) sensing Cys- branch of the PRT6 N-degron pathway. Overexpression of AtUCP1 results in increased stabilization of the N-degron reporter C-^{HA}GUS protein, which can be abolished entirely by the UCP1 inhibitor GTP. Transcriptome analysis of an Arabidopsis *ucp1* knockdown revealed the decreased expression of core hypoxic genes. Increased expression of UCP1, and increased C-^{HA}GUS stabilization co-occur at specific stages of development and when plants are submitted to specific abiotic stresses. In addition, stress-tolerance phenotypes observed in transgenics over-expressing UCP1 lines strongly resembled those observed for the N-degron pathway E3 ligase mutant *prt6-1*, whereas *ucp1* sensitivity to stresses resembled phenotypes observed for a pentuple mutant that removes activity of ERFVII transcription factors, known oxygen/NO sensing substrates of the PRT6 N-degron pathway. Enhanced stress tolerance phenotypes and gene expression observed in transgenics over-expressing UCP1 were removed in a genetic background without all five ERFVII proteins (*erfVII* pentuple mutant *UCP1ox*). Thus, the *UCP1ox* enhanced stress tolerance phenotype is dependent on stabilized oxygen/NO sening substrates of the PRT6 N-Degron pathway. The present work sheds a new light in UCP1 biological function in the regulation of the energetic and redox balance between the cytosol and mitochondria, and indicates that ERFVII stabilization is an important component in mitochondrial-nucleus retrograde signalling.

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SUBCELLULAR LOCALIZATION AND NITRIC OXIDE-SCAVENGING ACTIVITY OF PLANT HEMOGLOBINS

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Symbiotic hemoglobins provide O_2 to N_2-fixing bacteria within legume nodules. However, the functions of nonsymbiotic hemoglobins or phytoglobins (Glbs) are less defined. Three Glb classes can be distinguished based on phylogenetic and biochemical analyses and may coexist in plant tissues: class 1 Glbs have extreme O_2 affinity and are induced by hypoxia; class 2 Glbs have moderate O_2 affinity and are precursors of leghemoglobins; and class 3 have low O_2 affinity and high sequence homology with bacterial truncated hemoglobins. Immunogold labeling combined with confocal microscopy of Glbs tagged with GFP at the C-terminus was used to determine the subcellular localizations of Glbs in the model plants Arabidopsis and *Lotus japonicus*. To this end, we used overexpressing and knockout or silenced lines of Arabidopsis, performed quantitative immunolabeling, and monitored the GFP-tagged proteins in leaf cells and protoplasts. Recombinant proteins were used to examine NO scavenging *in vitro* and transgenic plants to show *S*-nitrosylation and other *in vivo* interactions with NO and ABA signaling. We found that Glbs occur in the nuclei, chloroplasts, and amyloplasts of both plant species, and in the cytoplasm of Arabidopsis cells. The proteins show similar NO dioxygenase activities *in vitro*, are nitrosylated in Cys residues *in vivo*, and scavenge NO in stomatal cells. The Cys/Ser mutation does not affect NO dioxygenase activity, and *S*-nitrosylation does not significantly consume NO. We demonstrate a crosstalk between Glbs and ABA on several grounds: Glb1 and Glb2 scavenge NO produced in guard cells following ABA supply; plants overexpressing Glb1 show higher constitutive expression of the ABA responsive/signaling genes *Responsive to ABA (RAB18)* and *Highly ABA-Induced 2* (*HAI2)*, and are more tolerant to dehydration; and ABA strongly up-regulates class 1 Glbs. We conclude that Glbs modulate NO and ABA signaling in crucial physiological processes such as the plant's response to desiccation.

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ROLE OF ROS SCAVENGING ENZYMES IN DESICCATION TOLERANCE IN FERNS

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Objectives

The desiccation tolerant (DT) fern species *Crepidomanes inopinatum* and *Loxogramme abyssinica* often grow together in the understory of KwaZulu-Natal Afromontane forests. *C. inopinatum* is a "filmy" fern that dries rapidly because it lacks a cuticle; by contrast *L.* abyssinica possesses a cuticle, and therefore dries slowly. It was predicted that DT in the fast-drying *C. inopinatum* relies mainly on constitutive mechanisms, while the slow-drying *L. abyssinica* depends more on inducible DT mechanisms. Here we tested whether increases in the activity of two ROS scavenging enzymes and the concentrations of soluble sugars occurs during a drying-rehydration cycle, mechanisms often suggested to contribute to DT tolerance in plants.

Materials and Methods

Plants were collected from Afromontane forest in KwaZulu-Natal. Vitality of the plants was tested using chlorophyll fluorescence, and the activity of the ROS scavenging enzymes superoxide dismutase (SOD) and peroxidase (POX), and concentration of soluble sugars were measured using standard techniques.

Results

Both species recover rapidly during rehydration after desiccation, confirming that both species are genuinely poikilohydric. In both species, slow desiccation increased the activity of the ROS scavenging enzyme SOD, and the concentrations of soluble sugars. Desiccation had little effect on the activity of the ROS scavenging enzyme POX, suggesting that maintenance of POX activity is a constitutive DT mechanism. The main difference between the two species was that de-acclimation occurred in the filmy fern; moist storage under cool dim light for a week reduced DT. By contrast, in the fern with a cuticle no de-acclimation occurred.

Conclusion

Taken together, results suggest that counter to our original hypothesis, inducible mechanisms, including the upregulation of ROS scavenging enzymes, occur even in filmy ferns that desiccate rapidly.

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PLANT REDOX RESPONSES TO WOUNDING AT ELEVATED CO_2 AND NITROGEN FERTILIZATION

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Increases in atmospheric carbon dioxide (CO_2) levels, predicted to occur before the end of the century, will impact plant responses to biotic stresses. In response to wounding, either mechanically or by chewing insect herbivores, rapid activates of the jasmonate pathway leads to downstream defense responses. In previous research, we observed an attenuation of the jasmonate burst when Arabidopsis thaliana plants grown at elevated CO_2 under high nitrate fertilization were wounded. Current research is ongoing to further understand the mechanism(s) underlying this suppression, focusing on changes in foliar reducing power and redox metabolites. We hypothesize that the decrease in photorespiration in this C3 plant when grown at elevated CO_2 levels affects foliar levels of reducing power and/or cellular redox potential that link with plant defenses. The rosettes of 4 week old Arabidopsis plants were mechanically damaged with scissors and NAD/NADH, NADP/NADPH and oxidized and reduced glutathione and ascorbate levels were measured over the next 45 minutes. Contrary to our hypothesis, resuts suggest a stronger link between these metabolites with nitrogen fertilization than atmospheric CO_2. Overall, results suggest that C3 plants may be more vulnerable to chewing insect herbivores under predicted future climatic conditions.

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CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 IN SALICYLIC ACID SIGNALLING

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Salicylic acid (SA) accumulation in many species results in plant growth inhibition. The vast majority of SA is synthesized in chloroplasts, as a derivative of shikimic acid, by isochorismate synthase 1 (ICS1). During investigation of SA signalling, a mutant of ICS1 has been described and named *sid2*. Isochorismate is also an intermediate in phylloquinone (vitamin K1) biosynthesis pathway. This molecule is a secondary acceptor in Photosystem I – mediated electron transport.

Previous studies revealed elevated SA level in *crk5* mutant plants. Moreover analysis of *CRK5* promoter sequence identified significant enrichment in *cis*-regulatory elements: W-box (TTGAC), which are recognized and bound by specific WRKY transcription factors, which regulate expression of genes involved in SA signal transduction.

In this study we demonstrate an effect of SA biosynthesis inhibition in *crk5*, obtained by *sid2;crk5* double mutant, which is able to revert lower biomass production phenotype. Based on source literature we decided to investigate susceptibility or tolerance of described genotypes to UV radiation. The level of the cell death after UV incident, exhibited in relative ion leakage, was almost two times higher in *crk5*, than in the wild type (WT) and *CRK5* overexpressing line (*CRK5:OE*). *sid2* and *sid2;crk5* showed higher resistance to the used dose of ultraviolet light. An analysis of chlorophyll b fluorescence uncovered, that regardless of conditions *sid2* mutants in both backgrounds showed decreased yield of photosystem II (Y(II)) and increased quantum yield of regulated (Y(NPQ)) and nonregulated (Y(NO)) energy dissipation.

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STUDIES ON THE ROLE OF VARIOUS RBOH ISOFORMS IN *ARABIDOPSIS THALIANA* UNDER AMMONIUM NUTRITION

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Cultivation using ammonium (NH_4^+) as the only source of nitrogen in the soil solution leads to the growth inhibition and developmental disorders known as „ammonium syndrome". Metabolism of reactive oxygen species (ROS) in the apoplastic space is one of the factors that can affect the cell wall structure and the processes involved in the plant growth and cell cycle. The main enzymes responsible for the intensified ROS generation in the apoplastic space are respiratory burst oxidase homologues (RBOHs). The plant RBOHs are localized in the plasma membrane and encoded by 10 genes in *Arabidopsis thaliana* (*RbohA*-*RbohJ*). Two RBOH isoforms (RBOHD and RBOHF), which are the most expressed isoforms in *Arabidopsis* leaves, were selected in the studies. For further investigation, also RBOHC, RBOHG, RBOHE, RBOHI isoforms were chosen.

The purpose of this study was to determine the influence of decreased expression of various RBOH isoforms (RBOHD, RBOHF, RBOHC, RBOHG, RBOHE, RBOHI) on ROS metabolism and the growth of *Arabidopsis thaliana* during ammonium nutrition.

The content of ROS, concentration of low molecular mass antioxidants and the growth abilities were analyzed in all tested mutants grown on NH_4^+ as the sole nitrogen source. Under ammonium nutrition, in RBOHE mutants, changes in the size of rosettes were observed in comparison to the control plants grown in the same conditions. Analysis of the root system showed a difference in the length and a number of lateral roots in tested mutants during the ammonium treatment. We showed that the decreased expression of RBOH isozymes changes apoplastic ROS metabolism during NH_4^+ treatment. Our results indicate that the expression of RBOHD and RBOHF isoforms is an important factor of ROS generation in the apoplastic space under ammonium nutrition.

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SINGLET OXYGEN PLAYS AN ESSENTIAL ROLE IN THE ROOT'S RESPONSE TO OSMOTIC STRESS

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The high osmotic potentials in plants subjected to drought stress can be mimicked by the application of high molecular weight polyethylene glycol. Here, we quantified the effects of exposure to polyethylene glycol on the growth of the main and lateral roots of Arabidopsis (Arabidopsis thaliana) seedlings. The effects on root growth were highly correlated with the appearance of singlet oxygen, as visualized using the singlet oxygen-specific probe singlet oxygen sensor green. The production of singlet oxygen was followed by cell death, as indicated by the intracellular accumulation of propidium iodide due to the loss of membrane integrity. Cell death began in the epidermal region of the root tip and spread in a dynamic manner to meristematic sections. In parallel, gene expression changes specific to the presence of singlet oxygen were observed. The accumulation of other reactive oxygen species, namely hydrogen peroxide, nitric oxide, and superoxide, did not correlate with cell death. In addition, both the singlet oxygen scavenger His and the lipoxygenase inhibitor salicylhydroxamic acid specifically inhibited singlet oxygen accumulation and cell death. These results suggest a light-independent, type-I source of singlet oxygen production. Serpin-protease interactions were used as a model to assess the possibility of vacuolar-type cell death. Osmotic stress induced the accumulation of complexes between the cytoplasmic serpin AtSERPIN1 and its cognate vacuolar proteases, indicating that vacuolar integrity was compromised. These findings imply that singlet oxygen plays an essential role in conveying the root response to osmotic stress.

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NITRIC OXIDE-MEDIATED REGULATION OF ETHYLENE METABOLISM DURING TOMATO FRUIT RIPENING

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A burst in ethylene production and respiratory rates is observed during climacteric fruit ripening, which coincides with the transcriptional and metabolic reprogramming linked to the organoleptic and nutritional changes distinctive of this developmental process. Accumulating evidence indicates that nitric oxide (NO) antagonistically interact with ethylene, repressing ripening and altering fruit quality traits. Here, we investigated the impacts of NO treatment on ethylene metabolism during tomato (*Solanum lycopersicum*) fruit ripening and its influence on the final fruit nutritional composition. Data revealed that the NO-triggered delay in tomato fruit ripening is associated with a significant reduction in ethylene emission at the climacteric phase, which was preceded by the accumulation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and repression of the central ethylene biosynthetic enzyme ACC oxidase (ACO) at both transcriptional and activity levels. The transcript abundance of genes involved in other steps of ethylene biosynthesis such as *ACC SYNTHASE* (*SlACS*) as well as some master regulators of ripening (*SlRIN* and *SlNOR*) were also negatively impacted by the NO treatment. The NO-induced down-regulation of ethylene biosynthesis was accompanied by the transcriptional repression of key carotenogenesis-related genes, leading to a marked reduction in ripening-associated accumulation of lycopene and β-carotene. In contrast, fruit tocopherol (vitamin E) profile remained relatively unchanged upon NO treatment, thereby indicating some specificity during the NO-mediated regulation of tomato fruit metabolism. Exogenous NO also promoted S-nitrosoglutathione reductase (GSNOR) activity, which may be associated with the rapid decline in NO content soon after the fruits were removed from the NO-enriched atmosphere. Protein S-nitrosylation and tyrosine nitration profiles were also monitored using the biotin-switch technique and anti-3-nitroY antibody-based Western blot, respectively, and the differences in the overall pattern of NO-mediated protein post-translational modification during tomato fruit ripening will be discussed.

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LIPOXYGENASE (LOX) IN SWEET PEPPER (*CAPSICUM ANNUUM* L.) FRUITS: IDENTIFICATION OF GENES AND ISOZYMES AND THEIR REGULATION BY NITRIC OXIDE (NO)

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Pepper fruit has remarkable agro-economical importance worldwide being also one of the fruits with higher vitamin C content [1]. Lipoxygenase (LOX) catalyses the oxygenation of polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acids being the first step in the biosynthesis of a large group of biologically active fatty acid (FA)-derived metabolites collectively named oxylipins. LOX is involved in diverse functions such as the biosynthesis of jasmonic acid (JA) as well as in the aroma and flavor production of plant tissues, among other. As part of the characterization of LOX activity from pepper plants we have analyzed the number of *LOX* genes in "sweet" pepper as well as the number of LOX isozymes present in the main organs (roots, stems, leaves and fruits) of pepper plants. The number of *LOX* genes in fruits was determined by a comparative analysis of RNAseq data previously obtained in green and red sweet peppers with the available information of "hot" red pepper and tomato fruits from the Uniprot database. Thus, we identified a total of eight *LOX* genes in pepper fruits with an identity with those from tomato between 54 to 91%. LOX activity was assayed in non-denaturing gel electrophoresis allowing identifying a total of seven LOX isozymes being the stems, the organs with the highest number of isozymes. Furthermore, the analysis of leaves from pepper plants exposed to low temperature (8 °C) for different time periods (1 to 3 d) also demonstrated a despair response of the LOX isozymes. Additionally, *in vitro* assays of fruit LOX activity in the presence of different reducing compounds and NO donors showed that this enzyme system was prone to be differentially regulated by those agents.

[1] Corpas *et al* (2018) **J Exp Bot** 69: 3449-3463.

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CADMIUM-INDUCED INTERDEPENDENCE OF ROOT AND LEAF RESPONSES RELATED TO GLUTATHIONE AND ETHYLENE IN *ARABIDOPSIS THALIANA*

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Due to its high toxicity and wide-spread occurrence, cadmium (Cd) has become an important environmental pollutant posing risk to human health and setting constraints on arable land. Even tough Cd is non-essential, it is readily taken up by plants leading to accumulation in the food chain and impaired plant growth. The latter, Cd-induced phytotoxicity, mainly arises from the depletion of important antioxidants like glutathione (GSH) leading to an oxidative challenge. The current project focusses on the interdependence of Cd-induced root and leaf responses with attention for GSH and ethylene, two key regulators of the responses to Cd stress. Whereas little is known about the root-shoot interdependence under Cd stress, previous results demonstrated that in roots of 5 µM Cd-exposed *Arabidopsis thaliana* plants GSH initially serves as a precursor of Cd-chelating phytochelatins[1]. Hence, GSH becomes rapidly depleted at the root level leading to an oxidative challenge. In the leaves, this depletion is not observed and GSH biosynthesis is stimulated. This stimulation is mediated by ethylene, which in its turn is enhanced in Cd-exposed roots and leaves[2,3]. To gain more insight into the timing of root and leaf responses related to GSH and ethylene in Cd-exposed plants, a kinetic exposure set-up was used and transcript levels, GSH concentrations and H_2O_2 levels were analysed. These results support the necessity of ethylene signalling for the stimulation of GSH synthesis. In leaves, this sequence of events is manifested at the transcript level, where transcriptional induction of ethylene synthesis precedes that of GSH synthesis. At the root level, however, this is more nuanced because the early GSH depletion triggers a first response including an early transcriptional induction of NADPH oxidases, which is maintained throughout 24 h of exposure. To further unravel the interaction between ethylene and GSH, biosynthesis mutants are studied and results will be presented.

1. Jozefczak, M., et al., Plant Physiology and Biochemistry, 2014. 83: p. 1-9.
2. Schellingen, K., et al., Environmental and Experimental Botany, 2015. 117: p. 1-11.
3. Schellingen, K., et al., BMC Plant Biology, 2014. 14(1): p. 1-14.

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ANTIMONY EFFECTS ON OXIDATIVE AND ANTIOXIDATIVE RESPONSES IN TOMATO PLANTS

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The alterations induced by the toxicity of antimony (Sb) in the roots and leaves of tomato (*Solanum lycopersicum*, L.) plants were determined. The plants were grown hydroponically with different concentrations of Sb (0.0, 0.5 and 1.0 mM), a heavy metal which reduces biomass production and growth, and relative water content (RWC). There was preferential accumulation of Sb in the roots, with the concentrations in the leaves being much lower. The accumulation of other mineral elements was also altered, Fe and Mn decrease in the roots, but on the contrary, Cu and Zn increased the concentrations both roots and leaves.
Chlorophyll a and b content declined, and the ratio chl a/chl b increase, but the carotenoid content remained unaltered. The photosynthetic efficiency decrease for 20% respect to the control. The total content of phenolics, flavonoids, and phenylpropanoid glycosides and polyphenol oxidase (PPO) activity are not altered.
The lipid peroxidation and $O_2^{\cdot-}$ generation increased only in roots while was similar to the leaves. The induced oxidative stress leads to a increase in the superoxide dismutase (SOD), ascorbate peroxidase (APX) and S-nitrosoglutathione reductase GSNOR activities, while the glutathione reductase (GR) and the inespecific-peroxidase (POX) activities were not altered. In contrast, the dehydroascorbate reductase (DHAR) activity decreased in the roots. The ascorbate (AsA) does not increase in roots but if it does in leaves, while the dehydroascorbate (DHA) increases in roots. The GSH decreases in roots, but in leaves enhanced. In contrary, the GSSG decreases in roots and practically does not alter in leaves. The ascorbate pool remained unaltered, whereas that of the glutathione pool decreased in roots and remained unaltered in leaves.
At the molecular level, Sb induces increases in the expression of APX and glutathione S-transferase (GST), but not in GR and CuZn-SOD, where decreases.

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COMBINED HEAVY METAL TREATMENT AFFECTS NITRO-OXIDATIVE STATUS OF RAPESEED AND SUNFLOWER ROOTS DIFFERENTLY

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The development of the root system is regulated by a complex and a diverse signalling network which, besides other factors, includes reactive oxygen (ROS) - and nitrogen species (RNS). The delicate balance of the endogenous signal system can be affected by various environmental stimuli, such as the excess of heavy metals (HMs). HM contamination of soils is growing problem, partly originated from agricultural processes. Many metal-polluted areas, especially where sewage as fertiliser is used, have high concentrations of more than one metal.

Our goal was to determine the nitro-oxidative status in the root system of rapeseed (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) subjected to combined HM treatment. The effect of model-sewage in two different layouts (control soil watered with model-sewage during seedling growth and soil pre-equilibrated with model-sewage) was simulated in rhizotron system by the use of the highest HM concentrations (Cd, Cr, Cu, Hg, Ni, Pb, Zn) legally allowed.

Combined HM treatment increased root tip metabolic activity of rapeseed, while decreased it in sunflower. In rapeseed's root tips, nitric oxide (NO) content changed in an opposite way depending on treatment-severity, while superoxide and peroxynitrite levels decreased, together with protein tyrosine nitration (PTN). In sunflower's root tips, both ROS and RNS levels decreased, but interestingly the milder combined HM treatment increased PTN significantly and lipid peroxidation was also detected after both treatments.

Results suggest species-specific molecular responses to combined HM stress.

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INVOLVEMENT OF CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASE 45 IN THE ROS WAVE

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Rapid accumulation and propagation of Reactive Oxygen Species (ROS) in plants in response to various stimuli, also known as the ROS wave, has been found to regulate different systemic signals. Although, some of the components of this signaling pathway have been identified, many are still unknown. Cysteine-rich receptor-like protein kinase 45 (CRK45) is a member of a membrane-bound receptor like kinase family. It was previously shown that *CRK45* transcription is regulated by ROS signaling and the protein itself might act as a sensor for ROS. In this study, we unveil the effect of CRK45 on the activation of systemic ROS signals in response to different stresses. Our approach consists on a novel live-imaging of wild type (WT) and *crk45* knockout mutants subjected to different local stress treatments. Using the novel live-imaging system, we detect the ROS in whole plants and can monitor the ROS wave initiation and progression in response to highlight illumination, injury or bacterial infection. Preliminary results show depletion of the ROS wave in *crk45* knockout mutants in response to highlight and bacterial infection while WT-like systemic signaling was observed following injury. These suggest that CRK45 is part of the ROS wave pathway in response to only to certain stresses; demonstrating the complexity of the ROS wave. Further studies are underway to determine the molecular function of CRK45 and other signaling genes in the ROS wave.

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NITRO-OXIDATIVE CHANGES IN EMBRYONIC AXES OF APPLE SEEDS SUBJECTED TO COLD STRATIFICATION

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Dormancy is a physiological state of the seeds in which most of the biochemical processes of the embryos are inhibited. Dormancy prevents seeds germination and allows them to survive unfavorable environmental conditions, thus it is an important adaptive feature. Apple (*Malus domestica* Borkh.) seeds are characterized by deep embryonic dormancy, which may be overcome by long-term (90 days) cold (5°C) stratification. The embryos isolated from stratified (non-dormant) seeds germinate well and the emerging seedlings do not have any developmental anomalies. During the seed stratification period the concentration of ROS and RNS fluctuates in apple embryos, leading to the dormancy alleviation. Despite ROS and RNS generation, we analyzed parameters of nitro-oxidative stress (total content of phenolic compounds, total antioxidant capacity of the tissues and RNA nitration level) in axes of apple embryos differing in the depth of dormancy resulting from the duration of seeds cold stratification. One of the markers of germination ability is the content of biotinylated proteins, which are stored in seeds during embryogenesis. Therefore we determined also the changes in the profile of biotinylated proteins in embryonic axes of seeds subjected to cold stratification for 7 to 40 days. We observed the link between the depth of seed dormancy and the content of biotin binding proteins, tissue antioxidant capacity and RNA nitration level.

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NITRIC OXIDE SIGNALING IN SALT TOLERANT AND SALT SENSITIVE VARIETIES OF SUNFLOWER DURING SEEDLING GROWTH

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Early phase of seedling growth, in general, exhibits high sensitivity to a variety of abiotic stress conditions, it is exposed to. Sunflower seedlings, in particular, have been observed to respond to salt stress, provided as 120 mM NaCl in the growth medium by altering extension growth in dark. Present investigations focus on interesting differences in Nitric oxide modulated biochemical signaling routes in salt tolerant variety (DRSH 1) and salt sensitive variety (PSH 1962) of sunflower (*Helianthus annuus* L.). Preliminary experiments highlight noteworthy impact of NO donar (Diethylenetriamine; DETA) and NO scavenger (Diethyldithiocarbamate; DETC) in control and salt stressed conditions on the extension growth and proliferation of roots as well as extension of hypocotyl in the salt tolerant and salt sensitive varieties of sunflower. Spatial distribution of and quantitative analysis of endogenous nitric oxide undertaken by confocal laser scanning microscopy and spectroflurometric analysis respectively, in the seedling roots and cotyledons at three developmental stages (2,4 ,6 day old). It shows a direct co-orelation of the degree of salt sensitivity/tolerance to NO signaling. Further detailed investigations on this track have shown crosstalk of NO with a wide variety of enzymatic and non-enzymatic proteins through their differential tyrosine nitration. The data so far obtained linking NO with salt sensitivity in sunflower seedlings is further supported through proteomic approach by LC-MS analysis.

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EXPRESSION PROFILING OF GENES ENCODING SUPEROXIDE-GENERATING RBOH IN PEPPER FRUITS: REGULATION BY NITRIC OXIDE (NO)

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Fruit ripening is a dynamic physiological process involving metabolic adjustments where ROS and RNS metabolism are involved. Respiratory burst oxidase homolog (Rboh), also called NADPH oxidase (NOX), is one of the main enzymatic sources of superoxide ($O_2^{\cdot-}$) radicals in plants. Previously, we have studied the activity of seven Rboh isozymes which were differently regulated during the ripening (green to red) of sweet pepper (*Capsicum annuum* L.) fruits [1]. In order to gain a deeper understanding of the *Rboh* gene regulation and function, we followed two approaches. First, identifying the number of *Rboh* genes in sweet pepper fruit and second analyzing how these genes are modulated by exogenous nitric oxide (NO) during ripening. To identify the number of *Rboh* genes in sweet pepper, we compared RNAseq data obtained from green and red sweet peppers [3] with the available information in Uniprot database for hot pepper and *Arabidopsis thaliana*. This allowed identifying 7 Rboh isozymes which share between 60 to 76% identity with Arabidopsis Rboh proteins. To study the potential NO involved in regulating *RBOH* genes' expression during fruit ripening, we designed a non-invasive treatment of sweet pepper fruits at the breaking point stage with exogenous NO gas [2,3]. We found 4 out of the 7 *Rboh* genes that were downregulated during fruit ripening which were also differently regulated by exogenous NO. This behavior was corroborated by quantitative PCR (qPCR). In summary, the present data provide new insight of *Rboh* genes in sweet pepper during ripening whose expression is regulated by exogenous NO.

[1] Chu-Puga et al. (2019) **Antioxidants** (Basel). 8(1). pii: E9
[2] González-Gordo et al. (2019) **J Exp Bot** erz136
[3] Palma et al. (2018) **Methods Mol Biol** 1747: 3-11

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FOLATE METABOLISM LINKS EPIGENETIC REGULATION TO REDOX HOMEOSTASIS

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The effect of climate change on plants is of great concern for natural ecosystems and agriculture. Plants can cope with changing environmental conditions through phenotypic plasticity. Epigenetic gene regulation involving DNA and histone methylation leads to phenotypic plasticity by changing in the accessibility of transcription factors that control cell identity. However, the molecular mechanisms that control DNA and histone methylation patterns in response to environmental stimuli are poorly understood. Previous studies in *Arabidopsis thaliana* illustrated that METHYLENTETRAHYDROFOLATE DEHYDROGENASE/CYCLOHYDROLASE 1 (MTHFD1), an enzyme of the cytosolic folate cycle, plays a crucial role in the regeneration of S-Adenosylmethionine (SAM), the methyl-donor for DNA and histone H3K9 methylation. Moreover, MTHFD1 contributes to the cytosolic redox status by the reversible formation of Nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH) (Gorelova et al., 2017). We have previously shown that a single EMS-generated nucleotide exchange in the $NADP^+$/NADPH-binding domain of the *mthfd1-1* mutant (R175Q) led to increased levels of SAM and its transmethylation byproduct S-Adenosylhomocysteine (SAH) along with genome-wide decreases in DNA and histone methylation (Groth et al., 2016). MTHFD1 therefore constitutes a potential link between epigenetic gene regulation and redox regulation. Our preliminary data supports this hypothesis since *mthfd1-1* mutants show less enzyme activity and therefore less NADPH production in the forward reaction than wild-type MTHFD1. Thus, the pool of enzymatic and non-enzymatic antioxidants, which rely on re-reduction by NADPH might be disturbed by an altered pool of reduction equivalents. For further verification of an impaired redox homeostasis, we have used roGFP2, a redox-sensitive version of the green fluorescent protein (GFP) to image the glutathione redox potential in *mthfd1-1* and wild type plants. Together with tissue staining using redox-sensitive compounds such as DAB and NBT to assess the formation of reactive oxygen species (ROS) our results support the involvement of MTHFD1 in cellular redox homeostasis. As reactive oxygen production and redox signaling are hallmarks of stress responses, we are now testing whether MTHFD1-dependent redox homeostasis is involved in the regulation of DNA and histone methylation during environmental stress.

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REGULATORY MECHANISMS OF THE PLASMA MEMBRANE ROS-PRODUCING ENZYMES, RBOHS, BY Ca^{2+} BINDING AND PHOSPHORYLATION AND THEIR EVOLUTION IN PLANTS

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Plant NADPH oxidases/Rbohs catalyze ROS production in the apoplast and play crucial roles in the regulation of development and stress responses. Biochemical analyses using a heterologous expression system have demonstrated that Rbohs from various land plant species are synergistically activated by binding of Ca^{2+} to the EF -hand motifs in the N-terminal regulatory region and phosphorylation. We compared the Ca^{2+} dependency among various isozymes as well as the effect of phosphorylation at specific sites. Unlike functional diversification of 10 Rbohs in Arabidopsis, only two isozymes, *MpRbohA* and *MpRbohB,* exist in a model basal plant *Marchantia polymorpha*. They share well-conserved regulatory domains as well as the catalytic domain universal in all Rbohs in land plants. Although they showed similar expression patterns in gametophyte thalli, their knockout mutants showed quite different phenotypes; *mprbohA*^{KO} exhibited severe defects in growth and development of thalli, while *mprbohB*^{KO} showed impaired stress-induced ROS production, suggesting their difference in biochemical properties. We determined Ca^{2+} dependency of the ROS-producing activity by heterologous expression, and Ca^{2+}-binding affinity by isothermal titration calorimetry. We also identified phosphorylation sites critical for Ca^{2+}-dependent activation, and analyzed the effect of phosphorylation on the Ca^{2+}-binding affinity. Results showed that phosphorylation at specific sites increase the Ca^{2+}-binding affinity and enhance Ca^{2+}-dependent activation, and Rbohs act as Ca^{2+} sensors in the ROS-Ca^{2+} signaling network. Based on phylogenetic analyses and biochemical characterization of the Rboh family, evolution of the ROS-Ca^{2+} signaling network in plants will also be discussed.

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SUPPRESSOR OF GAMMA RESPONSE 1 AND GLUTATHIONE: PARTNERS IN CRIME DURING THE CADMIUM-INDUCED OXIDATIVE STRESS RESPONSE IN *ARABIDOPIS THALIANA*

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Cadmium (Cd) indirectly increases reactive oxygen species (ROS) production in plants, possibly causing DNA damage. Upon perceiving DNA damage, cells activate the DNA damage response (DDR), regulating cell cycle progression, DNA repair and programmed cell death. A central regulator of the plant DDR is the transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1), a functional homolog of the mammalian p53. As previous results showed a significant induction of SOG1-regulated genes in leaves of Cd-exposed *Arabidopsis thaliana*, the main objective of this study was to further investigate the role of SOG1 in plant responses to Cd stress. Several parameters involved in the Cd-induced stress response were compared between leaves of wild-type (WT) and *sog1-7* mutant *A. thaliana* plants exposed to 5 µM $CdSO_4$ for 24 h, 72 h or 8 days.

The Cd-induced upregulation of pro- and antioxidative genes and genes involved in oxidative signaling observed in WT plants, was significantly less pronounced or absent in *sog1-7* mutants. Whereas Cd-induced H_2O_2 production was delayed in the mutant, the extent of lipid peroxidation upon prolonged exposure was significantly higher in mutant than WT leaves. The transcriptional upregulation of glutathione (GSH) biosynthesis and the increase in GSH concentrations typically observed after 72 h of Cd exposure, was absent in the mutant. To further unravel the interplay between SOG1 and GSH during Cd stress, the expression of SOG1-regulated genes was investigated in leaves of three GSH-deficient mutants. Results showed that the Cd-induced upregulation of these genes was absent in the mutant genotypes, indicating that the interplay between GSH and SOG1 functions in both directions and suggesting a role for GSH in the Cd-induced DDR. In conclusion, our results reveal SOG1 as a novel regulator of the Cd-induced oxidative stress response and show its reciprocal interaction with GSH in leaves of *A. thaliana*.

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PLASMAMEMBRANE BOUND CLASS III PEROXIDASES UNDER LOW OXYGEN STRESS

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The class III peroxidases are heme-containing proteins of the secretory pathway in higher plants with a high number of isoforms. So far, 158 peroxidases have been identified in the maize genome [1]. According to sequence analyses, ca. 50% of the class III peroxidases are membrane-bound, half of them are located in plasma membranes (PM) [2]. So far, several plasma membrane bound peroxidases in maize roots have been identified by our group (ZmPrx01, ZmPrx70, ZmPrx66, ZmPrx58, ZmPrx24, and ZmPrx81) [2+3]. Their functions may vary from different plant processes (etiolation, phytohormone metabolism, cell wall modification etc.) to biotic and abiotic stress response (wounding, pathogens, drought, heat shock and starvation) [4]. The role of class III peroxidases in adaptation to low-oxygen or flooding stress is still barely investigated. Proteomic data of PM proteins under flooding stress was done for soybean [5]. A previous study on leaves of 28 days old waterlogged maize plants showed an increased expression of *zmprx01* [6]. Proteomic analysis of those plants revealed two PM bound peroxidases (ZmPrx66 and ZmPrx42) with a significant increase after 28h of waterlogging [7].

This is the first study on class III peroxidases in maize roots under low oxygen stress. *Zea mays* L. var. Gelber Badischer Landmais was cultivated for 14 days and stressed with low oxygen. PMs were isolated with the aqueous polymer two-phase partitioning. A combination of gel-based methods, in vivo staining, expression analyses and activity measurements was performed to identify peroxidases and their potential function in low oxygen response.

References:
[1] PeroxiBase (26.03.2019)
[2] Lüthje S, Meisrimler CN, Hopff D, Möller B. Phylogeny, topology, structure and functions of membrane-bound class III peroxidases in vascular plants. Phytochemistry. 2011 Jul;72(10):1124-35. doi: 10.1016/j.phytochem.2010.11.023. Epub 2011 Jan 4. Review. PubMed PMID: 21211808.
[3] Lüthje S, Hopff D, Schmitt A, Meisrimler CN, Menckhoff L. *Hunting for low abundant redox proteins in plant plasma membranes*. J Proteomics. 2009 Apr 13;72(3):475-83. doi: 10.1016/j.jprot.2008.11.001. Epub 2008 Nov 11. Review. PubMed PMID: 19032993.
[4] Lüthje S, Martinez-Cortes T. *Membrane-Bound Class III Peroxidases: Unexpected Enzymes with Exciting Functions*. Int J Mol Sci. 2018 Sep 21;19(10). pii: E2876. doi: 10.3390/ijms19102876. Review. PubMed PMID: 30248965; PubMed Central PMCID: PMC6213016.
[5] Komatsu S, Hashiguchi A. Subcellular Proteomics: Application to Elucidation of Flooding-Response Mechanisms in Soybean. Proteomes. 2018 Feb 27;6(1). pii: E13. doi: 10.3390/proteomes6010013. Review. PubMed PMID: 29495455; PubMed Central PMCID: PMC5874772.
[6] Perrineau, FC. Regulation of class III peroxidases and respiratory burst oxidase homologs by biotic and abiotic stress in maize (*Zea mays* L.). Dissertation 2015
[7] Meisrimler CN, Buck F, Lüthje S. Alterations in Soluble Class III Peroxidases of Maize Shoots by Flooding Stress. Proteomes. 2014 Jun 26;2(3):303-322. doi:10.3390/proteomes2030303. PubMed PMID: 28250383; PubMed Central PMCID: PMC5302756.

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NITRIC OXIDE ENHANCES THE SUGAR METABOLISM AND MAINTAINS THE QUALITY OF RED RASPBERRY DURING STORAGE

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The influence of nitric oxide (NO) on the sucrose metabolism and the storage quality of red raspberry was investigated. In this study, red raspberry was treated with 5, 15, 30 µmol L^{-1} NO and 200 µmol L^{-1} c-PTIO solution for 2 min. The soluble solids contents (SSC), weight loss, respiratory rate, ethylene production, and the contents of anthocyanin, rutin, total phenolic, total flavonoid, $O_2^{\bullet-}$, $^{\bullet}OH$ of red raspberry were measured. And the effects of NO on sugar contents and the activities of enzymes associated with sugar metabolisms were investigated. The results showed that, compared with other concentrations, NO at 15 µmol L^{-1} could more effectively maintain the storage quality of red raspberries. NO at 15 µmol L^{-1} maintained high contents of anthocyanin, rutin, total phenol, and total flavonoids, and reduced the release of superoxide anion and hydroxyl radical; increased the enzymes activities of soluble sugars metabolism and regulated the composition of soluble sugars in red raspberries during storage. These results indicated that 15 µmol L^{-1} NO treatment could improve the antioxidant capacity, inhibit the release of respiratory and ethylene, and enhance the soluble sugar metabolism to maintain the storage quality of red raspberries.

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THE TRANSCRIPTOME PROFILE OF GERMINATING BARLEY IS AFFECTED BY PHYTOGLOBIN EXPRESSION VIA MODULATION OF NITRIC OXIDE

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To understand how the class 1 phytoglobin is involved in germination process via the modulation of nitric oxide (NO) metabolism, we performed the analysis of physiological and molecular parameters in the embryos of transgenic barley (*Hordeum vulgare* L. cv Golden Promise) plants differing in expression levels of the phytoglobin gene (Pgb+ and Pgb-) during germination. The germinating Pgb+ barley seedlings emitted much less NO than Pgb - plants, with the wild type plants showing intermediate levels of NO emission. The level of nitrosylated SH-groups in proteins decreased in Pgb+ embryos, in concert with the higher activity of *S*-nitrosoglutathione reductase, while a higher amount of *S*-nitrosylated proteins was recorded in Pgb- embryos. The increase of alcohol dehydrogenase transcripts and activity was observed in all tested samples, but more significantly in Pgb– embryos, which indicated that fermentation is involved in the anaerobic step of germination more actively when Pgb is downregulated. The upregulation of the genes encoding the components of the respiratory pathway non-coupled to proton pumping and ATP production, which include the genes of external NAD(P)H dehydrogenases (*NDB3*) and alternative oxidase (*AOX1a*) was observed at 3 h after imbibition in Pgb+ embryos. This might be related to the capacity of the external dehydrogenases and alternative oxidase to participate in the Pgb-NO cycle and regulate the levels of reactive oxygen and nitrogen species in germinating seeds. Furthermore, upregulation of the genes encoding succinate dehydrogenase and pyruvate dehydrogenase in Pgb+ embryos after radicle protrusion highlighted a substantial role of the Pgb-NO cycle in enhancing the tricarboxylic acid cycle for provision of metabolic intermediates and ATP at the early stage of germination. It is concluded that the operation of Pgb-NO cycle is essential for the maintenance of redox and energy balance in germinating seeds experiencing low internal oxygen concentrations before radicle protrusion.

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COMPARATIVE STUDY OF ANTIOXIDANT PROPERTIES IN LOWBUSH BLUEBERRY LEAVES: BIOCHEMICAL DIFFERENCES BETWEEN CONVENTIONALLY CUTTING AND SOMATIC EMBRYOGENESIS REGENERANTS

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In the plant kingdom, phenolic compounds represent an important group of secondary metabolites. Blueberries exhibit high antioxidant activities as they contain high levels of health-promoting phytochemicals. Due to the raised health awareness and significant levels of antioxidant activities, blueberries are becoming the small fruit crop of interest among plant breeders. Among 400-500 species in *Vaccinium* genus, blueberries exhibit comparatively higher antioxidant activities. There is plenty of evidence showing superoxide free radicals cause oxidative damage to lipids, proteins and nucleic acids which ultimately contributes to many health disorders, such as cancer, heart, vascular and neurodegenerative diseases. Antioxidants act as a promising factor to prevent these diseases by scavenging free radicals. Purpose of this study was to investigate the total phenolic and flavonoid contents, and antioxidant activity of the the lowbush blueberry *(V. angustifolium* Ait.) three wild clones from the leaf tissues of conventionally grown plants and their somatic embryogenesis counterparts. The chemical analysis has been performed on the extracts of one-year-old leaves. While total phenolics and flavonoids were determined by spectrophotometrically using the Folin-Ciocalteu and the aluminum chloride colorimetric methods, respectively, antioxidant activity was screened using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The higher level of antioxidants was detected in the somatic embryogenesis regenerants as compared to the conventionally cutting donor plants. Therefore, it is very important to study the effect of somatic embryogenesis on antioxidant properties to establish this regeneration pathway as a reliable option of commercial blueberry production.

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CYSTEINE-RICH RECEPTOR-LIKE KINASE CRK2 DIRECTLY REGULATES NADPH OXIDASE RBOHD IN *ARABIDOPSIS*

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Reactive oxygen species (ROS) production is a frequent result of receptor-like kinases (RLKs) signaling in a multitude of cellular processes. The role of ROS has shifted in recent years from toxic by-product to important signaling molecule. A major component of pathogen-associated molecular pattern (PAMP)-induced ROS production is plant NADPH oxidase, respiratory burst oxidase homolog D (RBOHD). However, the regulatory mechanisms targeting RBOHD to fine-tune ROS production are not fully understood. We have analyzed the function of CYSTEINE-RICH RLK 2 (CRK2) in controlling PAMP-triggered ROS production and immunity. CRK2 interacts with RBOHD *in vitro* and *in planta* and directly phosphorylates RBOHD on novel residues. Mutation of CRK2-targeted phosphorylation sites in RBOHD results in significantly altered ROS production in human embryonic kidney (HEK) 293T cells and *in planta*. Taken together, our work sheds light on the integration of different signaling components in the regulation of RBOH activity and provides evidence for an essential role of CRK2 as a novel regulator controlling ROS production activity during early defense response.

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MpRbohB-MEDIATED ROS PRODUCTION SYNERGISTICALLY ACTIVATED BY Ca^{2+} BINDING AND PHOSPHORYLATION BY MpCPK5 IS ESSENTIAL FOR POLAR TIP GROWTH OF RHIZOIDS IN *MARCHANTIA POLYMORPHA*

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ROS production by plant NADPH oxidases/Rbohs are synergistically activated by binding of Ca^{2+} to the EF-hand motifs and phosphorylation by several families of protein kinases, and thus have been implicated as a crosstalk point in the ROS-Ca^{2+} signaling network. Genetic redundancy of the components of the ROS-Ca^{2+} signaling network appears to be quite low in an emerging model liverwort *Marchantia polymorpha*, which has only 2 Rbohs, MpRbohA and MpRbohB, while 10 in Arabidopsis and 9 in rice. *MpRbohB* is expressed in various tissues including the apical meristematic zones and rhizoids of haploid thalli as well as reproductive organs. Multiple *mprbohB* mutant lines generated by CRISPR/Cas9 genome editing exhibited severe defects in polar tip growth of rhizoid cells mostly due to rupture of tips: almost completely impaired in the loss of function lines, which was partially recovered by exogenous treatment with H_2O_2, suggesting critical importance of MpRbohB-mediated ROS production in proper stiffness of the cell wall at the growing tip. Another m*prbohB* mutant harboring a 3-amino-acid deletion in one of the EF-hand motifs showed reduced Ca^{2+}-dependent ROS production and intermediate phenotype in rhizoid growth. The growing rhizoids showed oscillatory change in cytosolic Ca^{2+} concentration, and pharmacological inhibition of the Ca^{2+} dynamics also caused rupture of tips. One of the 6 Ca^{2+}-dependent protein kinases, MpCPK5, was found to phosphorylate and activate the ROS-producing activity of MpRbohB. Knockout of *MpCPK5* resulted in impaired tip growth of rhizoids similarly to *mprbohB.* Moreover, the phenotype of *mprbohB*^{KO} mutant was rescued by the wild type *MpRbohB*, but not by *MpRbohB* containing point mutations at the corresponding phosphorylation sites by MpCPK5 we identified. These results suggest that MpRbohB-mediated ROS production activated by both Ca^{2+} binding and Ca^{2+}-dependent phosphorylation by MpCPK5 is essential for the maintenance of cell wall integrity at the growing tips of rhizoids.

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NETWORK OF PATHWAYS MEDIATED BY LIGHT, ROS AND PHYTOHORMONES IN GERMINATING SEEDS OF *ARABIDOPSIS THALIANA*

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Light is one of the environmental stimuli's which together with endogenous factors (i.e. reactive oxygen species, ROS; phytohormones, ABA) are controlling processes occurring in seeds. Nevertheless, the possible mechanism of interaction between all these factors in the regulation of seed-related events remain unclear. In this study is postulated that during germination, light received by phytochromes (PHYs) induce a signal in cells of imbibed seeds, leading to changes in expression of genes involved in ROS/ABA metabolism and/or signal transduction, thereby contributing to dormancy alleviation and germination stimulation. One of the transcription factor responsible for the light-related modulation of genes expression is HFR1 (Long Hypocotyl in Far-Red). Taking this into account, the aim of this particular study was to elucidate the role of *HFR1* gene within the network of ROS/ABA/light induced signals in germinating *Arabidopsis thaliana* seeds. To verify the possible involvement of *HFR1* in this process, biological tests using dormant seeds of wild type (WT) Columbia-0 and *hfr1 Arabidopsis* mutant were conducted in various light conditions. In addition, the expression profile of *HFR1* gene was examined in samples obtained from germinating WT seeds imbibed on water and solution of ABA or diphenylene iodonium (DPI – an inhibitor of NADPH oxidase). To characterize the effect of light on the relative level of transcripts of genes related to ROS/ABA metabolism and/or signaling in germinating *Arabidopsis* seeds, the qRT-PCR analysis was performed. Moreover, *in situ* ROS localization in seeds germinating in different light conditions was performed using a NBT (nitroblue tetrazolium) staining and profiles of carbonylated proteins were identified by immuno-oxyblots. The obtained novel results indicated that *HFR1* may be involved in modulation of light-dependent seed germination in ROS/ABA-dependent manner. Moreover, the possible role of AGO1 protein in post-transcriptional regulation of expression of ROS-related genes during this process is also discussed (see poster by Kućko et al.).

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ROLE OF ARTIFICIALLY ADDED GLUTATHIONE AND ITS PRECURSOR IN INDUCING RESISTANCE TO *TOBACCO MOSAIC VIRUS* (TMV) AND A POWDERY MILDEW PATHOGEN (*EUOIDIUM LONGIPES*) IN SA-DEFICIENT TOBACCO

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The role of the plant hormone salicylic acid (SA) in disease resistance is well-known. SA production can be induced by biotrophic pathogens, activating different plant defense mechanisms. The role of glutathione (GSH) is also well known; primarily as an antioxidant but also as a plant stress signaling agent. SA-GSH interactions may play a pivotal role in plant disease resistance. SA production and resistance to phytopathogenic bacteria is increased in GSH overproducer tobacco (Ghanta et al., 2011, Planta 233:895-910.), while SA production is lowered in GSH deficient Arabidopsis plants (Han et al., 2013, Antiox. Redox Signal. 18:2106-2121.). Here we investigated how increased GSH contents influence defense responses to viral (TMV) and fungal (*E. longipes*) pathogens in SA deficient *Nicotiana tabacum* cv. Xanthi *nahG* tobacco. As a control we used *N. tabacum* cv. Xanthi that displays normal SA levels. Artificial elevation of glutathione levels in intact tobacco leaves was executed by injection with different concentrations of reduced glutathione (GSH) and (R)-(−)-2-Oxothiazolidine-4-carboxylic acid (OTC). TMV levels and the expression of a pathogenesis-related gene (*NtPR1a*) were detected by RT-qPCR while powdery mildew (*E. longipes*) detection was conducted by qPCR with specific primers. Our results showed that artificial elevation of GSH significantly increases resistance to TMV and *E. longipes* in SA deficient (nahG) tobacco. However, the expression of *NtPR1a* was not further induced by elevated levels of GSH in early stages of powdery mildew infection, suggesting that Nt*PR-1a* is not directly involved in these defense responses induced by GSH. In conclusion, GSH may complement the impaired disease resistance of SA deficient tobacco to TMV and *E. longipes*.

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A SINGLE AMINO ACID SUBSTITUTION OF ORANGE PROTEIN PROMOTES CAROTENOID ACCUMULATION IN SWEETPOTATO

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In plants, carotenoids play essential roles in light-harvesting processes and protect the photosynthetic machinery from photo-oxidative damage. In our previous studies, *Orange* gene (*IbOr*) from sweetpotato [*Ipomoea batatas* (L.) Lam] was isolated, which is involved in accumulation of carotenoids. IbOr protein with a holdase chaperone activity post-transcriptionally regulates phytoene synthase (PSY), an important enzyme in the carotenoid biosynthetic pathway. IbOr protects IbPSY stability, which leads to carotenoid accumulation and confers enhanced tolerance to heat stress at 47^{o}C and oxidative stress in *IbOr* transgenic sweetpotato plants. In addition, IbOr interacts with oxygen-evolving enhancer protein 2-1 (PsbP), an extrinsic protein of the oxygen-evolving complex (OEC) of PSII, and the holdase chaperone function of IbOr can protect PsbP from heat-induced denaturation. In this study, substitution of a single amino acid (R96H) in a wild-type IbOr shows dramatically enhanced carotenoid accumulation by up to 30-fold in the transgenic sweetpotato calli. To further explore the function of IbOr-R96H and its utilization to develop various industrial plants with enhanced carotenoid content and tolerance to abiotic stresses, transgenic sweetpotato plants overexpressing IbOr-R96H were successfully generated and are under characterization. Interestingly, IbOr-R96H transgenic sweetpotato plant showed enhanced tolerance to heat stress compared with IbOr-WT. We anticipate that IbOr-R96H transgenic sweetpotato plants will enhance production of carotenoids and various environmental stress tolerances for sustainable agriculture on marginal lands.

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TRANSGENIC SWEETPOTATO PLANTS OVEREXPRESSING TOCOPHEROL CYCLASE DISPLAY ENHANCED TOLERANCE TO ABIOTIC STRESSES

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Oxidative stress by reactive oxygen species (ROS) overproduced under various environmental stresses such as drought, high salt, extreme temperature significantly affects plant production. Tocopherols are lipophilic antioxidants to protect cellular components from oxidative stresses under stress conditions. Previously, we isolated five tocopherol biosynthesis genes from sweetpotato plants, including genes encoding 4-hydroxyphenylpyruvate dioxygenase, homogentisate phytyltransferase, 2-methyl-6-phytylbenzoquinol methyltransferase, tocopherol cyclase (IbTC), and g-tocopherol methyltransferase. In this study, transgenic sweetpotato plants overexpressing *IbTC* under the control of the CaMV 35S promoter (refer to as TC plants) via *Agrobacterium tumefaciens*-mediated transformation were generated to understand its functions in sweetpotato plants. Three (TC2, TC9, and TC11) plants with high transcript levels of *IbTC* were selected for further characterization. The predominant tocopherol in the sweetpotato tissues is determined as α-tocopherol by high performance liquid chromatography analysis. The α-tocopherol contents in leaves of TC plants increased 1.6 ~ 3.3 times higher than those of non-transformed (NT) plants. There was no significant change in tocopherol contents of storage roots between NT and TC plants. The TC plants showed enhanced tolerance to multiple environmental stresses including high salt, drought and oxidative stresses. TC plants maintained higher levels of photosystem II efficiency and chlorophyll contents reflecting their abiotic stress-tolerant phenotypes. These results suggested that *IbTC* gene could be useful for developing transgenic sweetpotato plants enriched with increased α-tocopherol levels and abiotic stress tolerances.

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MOLECULAER CHARACTERIZATION OF COLD STRESS-INDUCED *LIGNIN-FORMING PEROXIDASE* IN SWEETPOTATO PLANTS

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Peroxidases (PODs) are major enzymes regulating production and scavenging of reactive oxygen species (ROS) by catalyzing the redox reaction between hydrogen peroxide (H_2O_2) and various substrates. The secretory class Ⅲ PODs, plant-specific enzymes, play key roles in plant growth and development under biotic and abiotic stress, and are involved in lignification by polymerization of monolignols. However, the functions of PODs under cold stress in sweetpotato [*Ipomoea batatas* (L.) Lam] has not been elucidated in detail. Tropical-origin sweetpotato plants are sensitive to low temperature. Previously, a strongly cold-induced *Lignin-forming peroxidase* gene (designated *IbLfp*) was identified from transcriptome analysis in cold-treated storage roots of sweeptpotato. In this study, we isolated the *IbLfp* from sweetpotato for molecular characterization in transgenic sweetpotato plants. The expression level of *IbLfp* was the highest in fibrous roots than in other tissues of sweetpotato. Transcripts of *IbLfp* was highly increased under cold (4℃) and heat (45℃) conditions in both leaves and fibrous roots, whereas it was decreased under drought (30% PEG) and saline (200 mM NaCl) stresses in leaves. To investigate the physiological functions of the *IbLfp* in sweetpotato, transgenic sweetpotato plants overexpressing *IbLfp* by CaMV 35S promoter (refer to as LP plants). Three (LP2, LP3 and LP8) lines with high transcript levels of *IbLfp* were selected for further characterization. POD specific activity in LP plants showed 2.5~3.6 times higher than that in non-transgenic (NT) plants. LP plants showed enhanced tolerance to oxidative stress induced by methyl viologen (5 mM) using leaf discs. Further characterization of LP plants are under investigation in terms of stress tolerance of various abiotic stresses including low temperature and functions of *IbLfp* on lignification during the formation process of storage roots in sweetpotato. The storage ability of LP plants under low temperature will be also tested using the storage roots.

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A PLASMA MEMBRANE-BOUND PEROXIDASE FROM ZEA MAYS REGULATED BY INNER CLOCK AND ABIOTIC STRESS; ZMPRX85

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Class III peroxidases (EC 1.11.1.7) are heme-containing proteins of the secretory pathway with a high redundance and versatile functions. Soluble peroxidases had been characterized in numerous studies, whereas only a few studies exist on membrane-bound isoenzymes. At least four heme containing class III peroxidases have been detected in plasma membranes of primary roots of maize (*Zea mays* L.). In this work we focus on the purification and characterization of one of these peroxidases that could not be identified so far. The native enzyme was purified from highly enriched plasma membranes by a two-step protocol, cation exchange chromatography followed by size exclusion. Mass spectrometric analysis was used to identify several tryptic peptides of the purified peroxidase, identifying the protein as ZmPrx85. Tertiary structure of ZmPrx85 was modelled by a multitemplate approach. *In silico* analysis of its active center, calcium and heme binding-sites, post-translational modifications, substrate channels and surface charges were performed. Study of expression profiles of *zmprx85* revealed spatiotemporal organisation of the transcript in comparison to other plasma membrane bound peroxidases. Specific activities were measured with several substrates. ZmPrx85 showed the highest enzymatic activity against diaminobenzidine (DAB) and ferulic acid, although activities were detected against NADH, scopoletin, esculetin, coniferyl alcohol and guajacol. Enzyme kinetics were done for the two substrates that showed highest activity, DAB (K_m = 3684.618 µM) and ferulic acid (K_m = 138.312 µM). Promoter analyses of *zmprx85* revealed motifs that suggest a response to abiotic stress and regulation by circadian rhythm. To verify this prediction, analyses by qRT-PCR was performed. Besides a regulation by inner clock, short-term regulation of the peroxidase was found after treatment with chitosan, salicylic acid, wounding, sodium chloride and hydrogen peroxide. Based on these results a possible function of *zmprx85 i*n oxidative stress appears most likely.

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PURIFICATION AND CHARACTERIZATION OF A *PHYSCOMITRELLA PATENS* PEROXIDASE INDUCED BY OXIDATIVE STRESS

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Physcomitrella patens has been reported to be highly tolerant to different stress factors, such as drought, osmotic and saline stresses. This capacity is essential for survival in periods where water supply is a limiting factor. Peroxidases are heme-containing enzymes with a prominent role in stress responses, but only few studies of moss peroxidases induced upon stress have been reported. In this study we found a basic peroxidase that was induced upon hydrogen peroxide (H_2O_2) and salt (NaCl) treatments in *P. patens* leafy gametophore cultures. The addition of H_2O_2 and NaCl to the culture medium caused an increase in total peroxidase activity as well as in total phenolic compounds. Isoelectrofocusing revealed that the increase in total peroxidase activity may be due to the enhancement of a basic peroxidase, which was further purified and characterized. This peroxidase was isolated from a total protein extract in a three-step protocol, including ammonium sulfate precipitation, adsorption chromatography on Phenyl Sepharose and cationic exchange chromatography on SP Sepharose. The molecular mass for this protein was calculated to be 36.5 kDa according to MALDI-TOF and with a pI of 9.5. Specific activities were measured in the presence of different substrates, showing the highest enzymatic activity against coniferyl and sinapyl alcohols, as well as ferulic acid. Moreover, this purified peroxidase was subjected to tryptic digestion and identified according to its peptide fingerprint. Based on the presence of a signal peptide in its sequence, this peroxidase is directed to the cell wall, which together with the substrate preferences shown by this enzyme may indicate its possible specialized functions in the cell wall modification that is required in response to saline stress in order to maintain the water balance.

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CAN OXIDATIVE STRESS BIOMARKERS BE USED TO IDENTIFIY SPECIFIC RESPONSES BETWEEN NATIVE AND NON-INDIGENOUS MACROALGAE SPECIES?

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Equilibrium of marine environments is threatened by disturbances, such as over-exploration, pollution and climate change, that, by altering the marine ecosystems, are also responsible for the appearance and dominance of non-native species. Due to the competition with native species, and, consequently the eradication of these ones, the invasive species represent a serious problem to the marine ecosystems since they are one of the causes of the loss of marine biodiversity. In this sense, the main goal of this study was to assess the oxidative status of two macroalgae species – *Bifurcaria bifurcata* and *Sargassum muticum*, a native and a non-indigenous species, respectively, to understand the mechanisms behind their adaptation to a changing environment. To achieve this, four biological replicates were collected in Viana do Castelo (Portugal) in December 2018 and in February 2019, processed and several biochemical endpoints, such as photosynthetic pigments, hydrogen peroxide (H_2O_2), lipid peroxidation (LP), proline,glutathione, ascorbate, total phenols and thiols were evaluated. The results showed that *S. muticum* presented higher photosynthetic pigments content than *B. bifurcata*. Furthermore, regarding the oxidative damage, *B. bifurcata* presented higher H_2O_2 levels than *S. muticum*, while the opposite was observed for LP degree. The antioxidant response of these two species, evaluated by the contents of proline, glutathione, ascorbate, total phenols and thiols, demonstrated that the native species presented higher levels of these compounds than the non-indigenous one, except for the proline content, in which the opposite was observed. Overall, the results pinpoint that *B.bifurcata* and *S.muticum* employ different physiological mechanisms to overcome the environmental constraints, though more research is needed to fully understand their acclimation/adaptation mechanisms.

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CYSTEINE REDOX REGULATION OF CARBON METABOLISM IN *ARABIDOPSIS THALIANA*

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Posters

A common occurrence of abiotic and biotic stresses is the accumulation of reactive oxygen species (ROS) which leads to oxidative conditions. ROS homeostasis is controlled through a complex network of ROS production and scavenging enzymes. One way for proteins to sense redox changes is through the reversible oxidation of cysteine thiol groups such as disulfide bond, S-nitrosylation, S-glutathionylation. These post-translational modifications of cysteines act as molecular switches altering protein activity, subcellular localization and binding affinity.
Here, we describe how cysteines redox regulation influences the cellular carbon metabolism by regulating key metabolic enzymes in the mitochondria and the cytosol (TCA cycle and associated enzymes) **(1)**. We further decipher the redox regulation of two of these enzymes: cytosolic malate dehydrogenase (cMDH) and isocitrate dehydrogenase (cICDH) **(2,3)**.
Upon oxidative conditions, cMDH and cICDH undergo cysteine oxidation. On the one hand, cMDH1 experiences sulfur oxidation on specific cysteines and homodimerize through a C-terminal cysteine disulfide which protects cysteine (Cys330) from overoxidation. This inter-molecular disulfide formation switches cMDH1 from a non-covalent homodimer to a disulfide-linked homodimer, affecting the kinetics, the thermodynamic stability and represents an oxidized state which is reversible by thioredoxins **(2)**. On the other hand, cICDH cysteines are prone to S-nitrosylation and S-glutathionylation. We show that a specific S-glutathionylation on Cys363 inhibits cICDH activity, which is restored by glutaredoxins, suggesting a redox regulation of cICDH by glutathionylation **(3)**. Metabolic enzyme activities were studied in both ROS metabolism mutants (e.g. *cat2*) and in mutants affected in thiol reduction systems, suggesting a key role of the thiol reduction systems to protect plant metabolism from oxidation **(1, 2, 3)**.
Therefore, we propose that the redox switch of different metabolic enzymes may contribute to adapt the cell metabolism to environmental constraints.

(1) Daloso DM et al. (2015) Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria **Proc. Natl. Acad. Sci. USA**, 112(11):E1392-400. doi: 10.1073/pnas.1424840112.

(2) Huang J, Niazi AK et al (2018) Self-protection of Arabidopsis cytosolic malate dehydrogenase against oxidative stress. **J. Exp. Bot**., 69(14):3491-3505. doi: 10.1093/jxb/erx396.

(3) Niazi AK et al. (2019) Cytosolic Isocitrate Dehydrogenase is regulated by glutathionylation in Arabidopsis thaliana. **Antioxidants**. 2019 Jan 8;8(1). pii: E16. doi: 10.3390/antiox8010016.

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CHLOROPLAST ASCORBATE PEROXIDASE FUNCTION IN THE ABSENCE OF CYCLIC ELECTRON FLOW AROUND PHOTOSYSTEM I

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Chloroplasts are considered to be one of significant sources for ROS production under stressful conditions, such as high irradiance, in plant cells. However, loss-of-function mutants of ascorbate peroxidases (stromal sAPX and thylakoid membrane-bound tAPX), which are key players in H_2O_2 metabolism in chloroplasts, do not show stress sensitive phenotype. This is probably due to redundancy of peroxidases (such as peroxiredoxins) in chloroplasts and/or due to other systems that prevent ROS production. We herein focused on proton gradient regulation 5 (PGR5) protein, which is a key component of cyclic electron flow (CEF) around photosystem I, because balancing of ATP/NADPH ratio and activation of xanthophyll cycle through CEF are crucial for preventing ROS production. To investigate a functional relationship between APXs and PGR5, an Arabidopsis triple mutant lacking sAPX, tAPX and PGR5 was generated and analyzed.

Consistent with previous reports, *pgr5* mutant failed to induce non-photochemical quenching (NPQ, an indicator of xanthophyll cycle) and showed a light stress-sensitive phenotype, while *sapx tapx* double mutant behaved like wild type under high light. Compared with *pgr5* mutant, *sapx tapx pgr5* triple mutant had more prominent photo-bleaching phenotype under the stress. RNA-seq analysis suggested that expression of many marker genes for oxidative stress was highly up-regulated in the triple mutant. These findings indicate that chloroplast APXs are required for photo-protection in the *pgr5* background. Interestingly, the impairment of NPQ induction in *pgr5* was partially alleviated in the *sapx tapx* background due to the activation of xanthophyll cycle. Further genetic study showed that this alleviation requires the NDH-dependent CEF (alternative pathway). Taken together, our results indicate that chloroplastic APX function is compensated by the PGR5-dependent CEF, and provide a new insight into the role of H_2O_2 on the NDH-dependent CEF activity.

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ROLE OF REACTIVE NITROGEN SPECIES IN THE REGULATION OF AUTOPHAGY IN THE ROOTS OF *TRITICUM AESTIVUM*

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Objective

Catabolic process autophagy is considered as a non-specific stress response of cells, which contributes to the programmed death of individual cells and the survival of the whole organism. Reactive oxygen and nitrogen species are signaling molecules involved in autophagy in eukaryotic cells; however the data about NO-mediated regulation of autophagy in plants are scarce. The aim of present work is to unravel the possible link between NO, redox status and autophagy in plant cells.

Materials and Methods

Four-day old wheat seedlings were incubated in the solutions containing NO donors: spermine (SPM, 1, 10, 100 µM); sodium nitroprusside (SNP, 10 µM); KNO_2 (1 mM); KNO_3 (10 mM). Following parameters were analyzed: induction of autophagy, the levels of NO, redox status, energy status.

Results

Treatment of intact wheat roots with NO donors induced the formation of autophagosomes and increased the expression of autophagic genes (*ATG4*/*ATG6*/*ATG8*) but did not lower cellular viability. NO induced autophagy was accompanied by an increase in the level of H_2O_2 but not lipid peroxidation (except 100 µM SPM). These indicate the regulatory effect of NO donors in plants. Autophagy is an energy dependent process. Mild NO-donors (KNO_3 and SPM at low concentrations) slightly increases the mitochondrial membrane potential ($\Delta\Psi_m$) and upregulated the expression of SnRK1 subunits, while strong NO-donors (KNO_2, SNP and 100 µM SPM) decreased $\Delta\Psi_m$ and inhibited the rates of respiration.

Conclusion

In plants, an increased level of NO and corresponding accumulation of ROS induce the formation of autophagosomes and stimulate the expression of autophagic genes. NO-induced autophagy may be regulated by the changes in the functional activity of mitochondria.

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MELANINS IN LICHENS: TYPES AND REDOX PROPERTIES

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Objectives

Lichens are symbiotic photosynthesizing organisms possessing phenomenal stress tolerance. Among the factors, which contribute to high stress tolerance, secondary metabolites, including dark pigments melanins, play special roles. Melanization is widespread in lichens growing in environment with high levels of abiotic stress. Although much is known about the roles of melanins in UV protection of lichens, only scarce information is available about the structure and properties of lichen melanins. Aim of present investigation was to determine the types of melanins in different clades of lichens and to analyze their photoprotective and redox properties.

Materials and Methods

Lichens were collected from Afromontane forest in South Africa and from foliage forests in Norway and Russia. Extracted and purified melanins were analyzed for elemental analysis, the presence of structural groups by FTIR spectroscopy, UV-Vis absorption spectra and antioxidative activity.

Results

Main finding of present study is although the photobiont comprises only 5% of the lichen thallus, it determines the type of melanins produced by lichen mycobiont. Based on C/N ratio, lichens with N-fixing photobionts (cyanobacteria) synthesize eumelanins, while lichens with non-N-fixing photobionts (algae) produce allomelanins. Both types of melanins can be constitutively synthesized or induced by UV. Presence of aromatic and aliphatic groups was determined by FTIR spectroscopy. Photoprotective properties were shown by the UV absorption spectra. It was found that lichen melanins possess strong antioxidative activity confirmed by the reduction of DPPH radical. Interestingly, eumelanin from *Lobaria pulmonaria* displayed higher rates of DPPH reduction than allomelanin from *Cetraria islandica*.

Conclusion

Results presented here clearly demonstrate that melanised thalli are less sensitive to the adverse effects of high light and UV stress. Structural properties of melanins provide an effective defence mechanism against oxidative stress.

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IDENTIFICATION OF ASCORBATE PEROXIDASE GENE IN THE MOSS *DICRANUM SCOPARIUM* (Hedw)

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Objective

Evolutionary studies of mosses made them attractive model systems for investigating reactive oxygen species (ROS) production and defense-related antioxidative enzymes. Ascorbate peroxidase (APX; EC 1.1.1.11) is a heme peroxidase that catalyzes the conversion of H_2O_2 into water using ascorbate as a specific electron donor. The limited data available on APXs of mosses suggest that they can be involved in the response of mosses to abiotic stresses. Information on the APX genes in mosses is scarce, and enzyme activity is poorly studied. Therefore, the aim of this work was to study the role of APX in abiotic stress tolerance of moss *Dicranum scoparium*.

Materials and Methods

Ascorbate peroxidase activity in the crude extract of *D. scoparium* was determined spectrophotometrically after a rehydration-dehydration-rehydration cycle and temperature exposure of -20 °C, + 30 °C and + 50 °C. PAGE separation of proteins was conducted in a non-denaturing condition and addition of 1% SDS. Gene cloning and sequencing was performed according to Senger reaction. Identification of APX gene sequence and bioinformatic analysis of the gene structure was performed with Vector NTI suite 9 and BLAST program on the NCBI server.

Results

Increase in APX activity of *D. scoparium* was observed after 2 d of rapid (silica gel) and slow ($CaCl_2$) desiccation. APX activity decreased generally after temperature exposure. Electrophoretic protein separation showed different isoforms of APX. Ascorbate peroxidase gene of *D. scoparium* showed 91% and 79.9% homology with the APX genes of *Grimmia pilifera* and *Physcomitrella patens*. Also, 64-80% identity of APX gene of *D. scoparium* and APX genes of higher vascular plants was found.

Conclusion

APX is an evolutionarily conserved enzyme that plays important roles in antioxidative protection of higher vascular and non-vascular plants during biotic and abiotic stresses.

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THE EFFECT OF ZINC OXIDE NANOPARTICLES ON ROS AND RNS METABOLISM OF BRASSICA ROOTS

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Zinc oxide nanoparticles (ZnO NPs) have unique chemical and physical traits, making them useful both in industry and agriculture. The application of ZnO NPs intensified in the past years, supporting the importance to evaluate the ZnO NPs effect on different plant species.

Our experiments compared the effects of 6 nm ZnO NPs on two agriculturally relevant plant species: indian mustard (*Brassica juncea L. Czern. cv. Negro Caballo*) and rapeseed (*Brassica napus L. cv. GK Gabriella*). Plants were grown on filter paper for five days in Petri dishes containing 0 (control), 25 or 100 mg/L ZnO NPs. We examined the effects of ZnO NPs on root growth, used fluorescent probes to evaluate the levels of reactive oxygen (ROS) and nitrogen species (RNS). Protein tyrosine nitration as a marker for nitrosative stress was detected by western blot using anti-nitrotyrosine.

The 25 mg/L ZnO NP treatment seemed to be beneficial for root growth, increased the length and fresh weight of the primary root. In contrast, 100 mg/L ZnO NP had a significant inhibitory effect. Zinc ion levels increased in roots as a response to both ZnO treatments and this was associated with a decreased viability of the root meristem cells. The ROS (hydrogen peroxide, superoxide anion) and RNS (nitric oxide, peroxynitrite) levels were modified by ZnO application, resulting in an altered protein tyrosine nitration pattern.

Our results suggest, that nitro-oxidative processes contribute to ZnO NPs-induced phytotoxicity.

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NICKEL-INDUCED ROS AND RNS IMBALANCE IN *BRASSICACEAE*

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Nickel (Ni) is a trace metal emitted into the environment from both natural and anthropogenic sources. Elevated concentrations of Ni cause symptoms like inhibition of plant growth, photosynthesis, seed germination, sugar transport and induction of chlorosis due to disruption of iron metabolism. Our knowledge about the molecular mechanisms of Ni phytotoxicity is incomplete, therefore this study aims to examine the nickel-induced alterations of reactive oxygen (ROS)- and nitrogen species (RNS) levels and protein nitration in non-accumulator *Arabidopsis thaliana* (Col-0) and accumulator *Brassica juncea (L. Cern*. cv. Negro Caballo). Plants were grown on Murashige-Skoog media supplemented with 0, 25, 50, 75 or 100 µM nickel chloride for 7 days.
Nickel-exposed *Brassica juncea* seedlings showed better tolerance, slighter changes in root hydrogen peroxide, superoxide, peroxinitrite and nitric oxide levels compared to Col-0 *Arabidopsis.* Nickel caused differently modified callose, pectin contents and flavonol (kaempferol, quercetin) levels in both species. The RNS-induced protein nitration was also influenced by nickel in the examined species. This preliminary study suggests Ni-induced nitro-oxidative stress which is associated with the capability of plant species to tolerate nickel.

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SWEET PEPPER FRUITS CONTAINS AN ATYPICAL CATALASE MODULATED BY REACTIVE NITROGEN SPECIES

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Catalase (CAT; EC 1.11.1.6) is a peroxisomal metalloenzyme considered as part of the first antioxidative barriers against reactive oxygen species (ROS), due to its ability to decompose H_2O_2. In plants, CATs have been described as homotetramers of about 220-240 kDa with subunits sizes ranging 55-57 kDa. However, using sweet pepper (*Capsicum annuum* L.) fruits as model, an average native molecular weight of 125 kDa was determined by non-denaturing PAGE at different acrylamide concentrations, and by gel filtration chromatography through an FPLC system. By Western blotting analysis, using an antibody raised against plant catalase, a single immunoreactive band of 55 kDa was detected. Accordingly, catalase from pepper fruits seems to be an atypical homodimer. Isoelectric focusing and specific catalase activity staining provided one unique isoenzyme with an isoelectric point of 7.4, what is also atypical considering other plant CATs reported so far.

Regarding to the metabolism of pepper, it was found that in ripe red fruits the catalase gene expression and the enzyme activity decreased with respect to immature green fruits, and this pattern was parallel to the catalase protein content observed after both iTRAQ approach and immunoblotting analysis. This behavior on enzyme activity during ripening could be due to post-translational modifications undergone by catalase as consequence of both nitration and *S*-nitrosation processes promoted by reactive nitrogen species (RNS). *In vitro* assays of pepper samples incubated with different chemicals including SIN-1 (a peroxynitrite donor), and DeaNONOate and *S*-nitrosoglutathione (GSNO) as NO donors, provoked significant decreases of catalase activity. Also, assays with the biotin-switch technique, trypsin digestion and further mass spectrometry (QTOF) provided a peptide located near the N-terminus which could be potentially *S*-nitrosated. These results highlight the cross-talk between NO and antioxidants in pepper fruits at ripening.

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KEY PLAYERS IN REDOX HOMEOSTASIS DURING THE LEGUME – RHIZOBIA SYMBIOSIS

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Reactive Oxygen Species (ROS) are signaling molecules controlling various fundamental processes. During the nitrogen-fixing symbiosis (NFS) between *Medicago truncatula* and *Sinorhizobium meliloti*, ROS play a crucial role in different stages of the interaction. Several lines of evidence showed that *M. truncatula* respiratory burst oxidase homologue genes (*Rbohs*) contribute to ROS synthesis during NFS. In parallel, *S. meliloti* possesses an active enzymatic (catalase, superoxide dismutase) and non-enzymatic (glutathione) antioxidant defense participating to its hosting in root nodules, the symbiotic organs. In this context, our goal was to determine ROS signatures during NFS.
For this, we used two redox biosensors consisting of a redox-sensitive green fluorescent protein (roGFP2), genetically fused to Orp1 (specific to hydrogen peroxide, H_2O_2) or Grx1 (related to glutathione redox state). These biosensors exhibit excitation maxima at 400 nm (oxidized form) and 490 nm (reduced form) which permits ratiometric measurements. We introduced these biosensors in both symbiotic partners and fluorescence signals were analyzed by spectrofluorimetry and confocal microscopy.
We first validated the functionality of these biosensors by treating cells with reductant (DTT) and oxidant (H_2O_2) compounds. We strengthened our results by performing *in vivo* H_2O_2 and redox imaging in *M. truncatula* mutants (in NADPH oxidases or in symbiotic receptors) and *S. meliloti* mutants impaired in antioxidant defense (catalase and glutathione synthetase). Acquired signals in the mutants confirm the capacity of biosensors to perceive intracellular redox modifications in the two symbiotic partners. Our preliminary results clearly highlight differences in both symbiotic partners during the different steps of symbiosis (i.e. infection threads, nodules).
In conclusion, we provide new insights in redox signaling in the NFS. The use of biosensors in the symbiotic partners opens new possibilities to dissect plant and bacteria H_2O_2 dynamics and redox regulation, including NADPH oxidase-mediated ROS signaling.

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S-NITROSOGLUTATHIONE REDUCTASE (GSNOR) IN 8-NITRO-CGMP-DEPENDENT SIGNALLING PATHWAYS OF ABA-INDUCED STOMATAL CLOSURE

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8-nitroguanosine 3',5'-cyclic monophosphate (8-nitro-cGMP) has been recently identified as an unique electrophilic intermediate involved in intracellular redox signalling. In animal cells, 8-nitro-cGMP is formed from the guanine nucleotide pool by a combined action of nitric oxide (NO), reactive oxygen species and guanylate cyclase. Although *in vivo* 8-nitro-cGMP shows certain biological activities closely resembling its analogue cGMP (e.g. vasodilation), its regulatory functions are mediated mainly by chemical interactions with protein thiols resulting in a novel post-translational modification termed S-guanylation. In *A. thaliana*, 8-nitro-cGMP was reported to mediate NO-dependent signalling pathways controlling abscisic acid (ABA)-induced stomatal closure; however, its derivative 8-mercapto-cGMP (8-SH-cGMP) was later shown as the active component of hydrogen sulphide (H_2S)-mediated guard cell signalling.

The main objective of our study was to uncover the role of protein S-nitrosation and S-nitrosoglutathione reductase (GSNOR) within regulatory pathways of 8-nitro-cGMP in stomatal closure. We used a microscopy analysis of epidermal stripes of *Nicotiana tabacum* cv. Xanthi to analyse effects of 8-nitro-cGMP and 8-SH-cGMP on tobacco stomata. Using a pharmacological approach, we have confirmed that similar signalling mechanisms previously described in *A. thaliana* guard cells operate also in tobacco, with calcium ions, cyclic ADP ribose and H_2S acting down-stream of 8-nitro-cGMP. Interestingly, an increased NO production was detected in tobacco guard cells treated with both 8-nitro-cGMP and 8-SH-cGMP suggesting their plausible targets within guard cells include also proteins involved in NO metabolism. Immunohistochemical analysis indicated that 8-nitro-cGMP and 8-SH-cGMP induced stomata closure was preceded by GSNOR induction. Furthermore, ABA-induced stomata closure was inhibited in a concentration-dependent manner by GSNOR inhibitor N6022, which could be reversed by dithiothreitol treatment. These findings underscore the important role of cGMP-independent signalling pathways of NO in stomata regulation mediated by protein S-nitrosation.

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THE SHARE OF CELL-WALL PEROXIDASES IN APOPLASTIC ROS PRODUCTION IN RESPONSE TO AMMONIUM TOXICITY

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Acute apoplastic ROS production occurs upon exposure of plants to environmental stresses. Just as prolonged application of ammonium (NH_4^+) as the sole nitrogen fertilizer, can be considered a major stress factor leading to growth suppression of most cops. The main sources of ROS in the extracellular space are the respiratory burst oxidase homolog (RBOH) and cell wall peroxidases (POX). However, POX have an additional function, their activity leads to cross linking of carbohydrates and stiffening of the cell wall. The aim of this study was to determine the role of cell wall POX in the appearance of the ammonium toxicity syndrome.

We showed that when *Arabidopsis thaliana* was grown on NH_4^+ as the sole nitrogen source H_2O_2 levels were elevated in the extracellular space. Further, the activity and protein level of POX was determined in the cell-wall fraction or apoplastic washing fluids and expression of selected POX isoforms was analysed. The activity of apoplastic POX participating in the oxidative cycle was higher in NH_4^+ fed plants. Also the peroxidative activity of POX was elevated, together with a higher phenolics pool in the cell wall fraction.

It can be concluded that the induction of apoplastic POX in response to NH_4^+ treatment can on one hand power the ROS burst and on the other hand, the activity of cell-wall POX can reduce cell wall extensibility and restrict growth of NH_4^+ grown plants. Therefore, the divergent activity of extracellular POX may contribute to growth inhibition under NH_4^+ toxicity.

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SIGNS OF AMMONIUM TOXICITY IN ARABIDOPSIS THALIANA

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Even though ammonium (NH_4^+) is an excellent nitrogen source for plants, in high doses it can lead to growth inhibition. The cause for the ammonium toxicity syndrome may be the formation of toxic metabolic by-products downstream of NH_4^+ assimilation. For instance high respiratory rates can provoke the generation of reactive oxygen species (ROS). While high glycolytic activity may result in the accumulation of methylglioxal (MG), which is considered a biomarker for abiotic stress adaptation. The aim of this study was to determine symptoms of toxicity in response to ammonium nourishment.

We showed that when *Arabidopsis thaliana* was grown on NH_4^+ as the sole nitrogen source elevated rates of O_2^- and H_2O_2 were produced in leaves. As a consequence high levels of oxidised proteins were detected in tissues indicating oxidative stress. Moreover, a strong induction of MG formation was noticed in tissues of NH_4^+ treated plants leading to dicarbonyl stress. Accordingly high levels of MG-derived advanced glycation end products were detected in proteins. The damage to proteins could not be reduced, despite high proteolytic enzyme activity in NH_4^+ fed plants. It can be concluded that the damage of crucial proteins may be a major factor affecting cellular metabolism under NH_4^+ toxicity and reduce growth of plants.

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ROLE OF NITRIC OXIDE IN IMPROVING SEED GERMINATION AND ALLEVIATION OF COPPER INDUCED PHOTOSYNTHETIC INHIBITION IN INDIAN MUSTARD

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Heavy metal stress limits crop production through its effects on seed germination and photosynthesis. Earlier reports suggest that nitric oxide (NO) as a signaling molecule plays an important role in heavy metal stress tolerance. The present study was carried out to study the effect of NO application in the alleviation of copper (Cu) induced negative effects on seed germination and photosynthesis of mustard (*Brassica juncea* L.) plants. Pretreatment with sodium nitroprusside (SNP), an NO donor significantly improved seed germination and alleviated Cu-accrued oxidative stress in *B. juncea* seeds. However, in the absence of NO, Cu showed a higher reduction in seed germination rates. Further, NO pretreatment increased activities of antioxidant enzymes and sustained the lower level of H_2O_2 and thiobarbituric acid reactive substances (TBARS) thereby elevated the antioxidative capacity in Cu-exposed *B. juncea* seeds. NO pretreated seeds also tended to retain higher amylase activities required for the proper seed germination than that of the control without SNP pretreatment. The present research also evident that NO mitigates Cu toxicity through the improved antioxidant system, photosynthetic efficiency and reducing Cu induced accumulation of reactive oxygen species accompanied by a reduction in lipid peroxidation at the vegetative phase of the mustard plant. Based on the results it can be concluded that NO modulated the activities of antioxidant enzymes, improved amylase activity and thereby improved the germination of *B. juncea* seeds under Cu stress and NO efficiently counteracts Cu toxicity by up-regulating antioxidant enzymes and reduced ROS accumulation resulting in higher photosynthetic rate.

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IDENTIFICATION OF NO-DEPENDENT SIGNALLING IN PLANT RESPONSE DURING Cd STRESS

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Cadmium (Cd) is a toxic non-essential heavy metal, which is able to enter plants and is therefore able to enter the food chain thus creating a main environmental and health problem worldwide. Understanding the mechanisms which plants use to get over Cd stress would allow the production of plants with greater Cd uptake potential for phytoremediation to recover soil efficiency in self-sustaining ecosystems (Romero-Puertas et al., 2019). Nitric oxide (NO) is a signalling molecule that has been involved recently in the response to Cd stress although its function is not well unknown (Besson-Bard et al., 2009). In this study, we have examined both in-house and public data sets derived from the profiling of Arabidopsis gene expression in mutants and treatments related to NO and Cd in order to identify a data set of genes regulated by NO in plant response to Cd which will enable us to gain a deeper understanding of the role played by this molecule as regulator of cellular responses to adverse conditions resulting in plant acclimation and resistance.

Besson-Bard A et al. Plant Physiol, 149 (2009): 1302–1315.
Romero-Puertas et al., Environ Exp Bot, 161 (2019): 107-119.

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THE CHAPERON-LIKE PROTEIN CDC48 REGULATES ASCORBATE PEROXIDASE IN TOBACCO

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Cdc48, a molecular chaperone-like protein conserved in different kingdoms, is a member of the AAA+ family contributing to numerous cellular processes in mammalian and yeasts. The functions of Cdc48 plant orthologues are less understood but Cdc48 emerges as a new factor of immunity. Previously, we highlighted that NtCDC48 is specifically S-nitrosylated in tobacco cells undergoing an immune response induced by cryptogein, a protein secreted by the oomycete *Phytophthora cryptogea*, which triggers, among other things, a hypersensitive response, a programmed cell death (PCD) confining pathogens to restricted necrotic lesions. We so investigated the function of NtCdc48 in cryptogein-treated tobacco cells and highlighted that it accumulates in elicited cells. Moreover, the cryptogein-induced PCD is accelerated in a cell line overexpressing NtCdc48. An immunoprecipitation-based strategy followed by mass spectrometry analysis led to the identification of about hundred NtCdc48 partners. Among them, the redox regulator cytosolic ascorbate peroxidase (cAPX) was identified. We therefore studied its regulation by NtCdc48 during cryptogein-induced immune response. We confirmed the interaction between NtCdc48 and cAPX, and showed that it occurs in the cytoplasm and independently of immune response. Then we provided evidences that cAPX accumulation was modified in cells overexpressing NtCdc48, a process that was shown to involve post-translational modification of cAPX. Furthermore, cAPX activity as well as the levels of both reduced (GSH) and oxidized glutathione (GSSG) and GSH/GSSG ratios are strongly affected in cells overexpressing NtCdc48 during cryptogein elicitation. Since a decrease in cAPX activity was also observed in response to heat shock in the cells overexpressing NtCdc48, the regulation of cAPX by NtCdc48 is not specific to the immune response.

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REGULATION OF PEROXULE FORMATION AND PEROXISOME PROLIFERATION BY PEROXISOMAL ROS SOURCES

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Peroxisomes are highly dynamic and metabolically active organelles which play an important role in cellular functions, including the reactive oxygen species (ROS) metabolism. Peroxisomal dynamics, such as proliferation and movement, have been associated with reactive oxygen species (ROS) in plant cells [1].To determine whether peroxules and peroxisomal proliferation are regulated by peroxisomal sources of ROS, we analysed the formation of these structures and the number of peroxisomes in different Arabidopsis mutants showing altered ROS production associated with peroxisomal photorespiration (glycolate oxidase, GOX) and fatty acid β-oxidation (Acyl CoA oxidase, ACX). Confocal analysis of *gox2xpx-ck,* showed that H_2O_2 from this source differentially regulates peroxisome proliferation, but did not affect peroxules formation in response to Cd. However, *ACX1* deficiency interferes with peroxule formation but does not significantly alter peroxisome proliferation in response to Cd. Analysis of *PEX11a* and *PEX11e* expression, in the different mutants suggests that, in addition to transcriptional regulation of PEX11a and e, post-transcriptional modifications of these proteins may be involved in the regulation of peroxule formation and peroxisomal population. We therefore conclude that peroxisomes can discriminate between different ROS sources, which could play an important role in the perception of specific stimuli.

[1] Rodríguez-Serrano et al. (2016) Plant Physiol 117, 1665-1674

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NITRIC OXIDE INCREASES SULFUR ASSIMILATION TO REVERT THE GLUCOSE-MEDIATED PHOTOSYNTHETIC REPRESSION IN WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALT STRESS

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Salt stress is one of the major abiotic stress factors that negatively impacts growth and development of agricultural crops worldwide. The influence of salt stress on plants is eminent because of drastically changing climatic conditions. Salt stress primarily causes disturbance in nutrients availability and osmotic stress. The accumulation of excess ions in plants growing under salinity results in formation of excessive reactive oxygen species (ROS), which accelerates damage to lipids, proteins and nucleic acids in cells. The accumulation of glucose (Glu) as an avoidance mechanism further aggravates the negativity of salt stress on photosynthetic capacity of plants. Nitric oxide (NO), a gaseous plant hormone is known to involve in several reactions in plant cells under normal and abiotic stress conditions. This study reports the involvement of NO in reversal of glucose-inhibited photosynthetic capacity in presence or absence of salt stress and elucidates the association of NO effects with sulfur (S) assimilation in wheat (*Triticum aestivum* L.). Nitric oxide (50µM NO) and 6% Glu applied to plants reduced the effect of 100mM NaCl on photosynthetic characteristics that was associated with increased S assimilation. The independent treatment of NaCl or Glu inhibited the photosynthetic performance of plants, but NO supplementation in plants treated with Glu and grown under salt stress exhibited restoration of photosynthetic characteristics through reduction of oxidative stress (content of H_2O_2 and TBARS). Plants treated with NO in presence of 6% Glu under salt stress exhibited increased S assimilation capacity measured as increased ATP-sulfurylase (ATP-S) activity and S accumulation, leading higher production of cysteine (Cys) and reduced glutathione (GSH) and enzyme of ascorbate-glutathione cycle that NO bears potential in increasing S assimilation leading to reduced oxidative stress to revert the Glu-mediated repression of photosynthesis. Thus, the modulation of NO in plants grown under salt stress may be an important tool to counteract the adverse effects of accumulated Glu under salt stress.

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ROS-INDUCED CA AND K FLUXES CORRELATE WITH SALT TOLERANCE IN CEREALS: TOWARDS THE CELL-BASED PHENOTYPING

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Main objective(s) of the study:
Salinity stress-induced ROS production and associated oxidative damage is one of the major factors limiting crop production in saline soils. However, the causal link between ROS production and stress tolerance is not straightforward as ROS may also play an important signaling role in plant adaptive responses

Materials and methods:
The causal relationship between salinity and oxidative stress tolerance in two cereal crops—barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) was investigated by measuring the magnitude of ROS-induced net K^+ and Ca^{2+} fluxes from various root tissues using non-invasive microelectrode ion flux estimation (MIFE) method and correlating them with overall whole-plant responses to salinity.

Results:
Association between flux responses to oxidative and salinity stress tolerance was found to be highly tissue specific and dependent on the type of ROS applied. A significant positive correlation was found for the magnitude of ROS-induced K^+ efflux and Ca^{2+} uptake and the overall salinity tolerance when oxidative stress was administered via H_2O_2 treatment, but only for mature zone and not the root apex. No correlation was found between root responses to hydroxyl radicals. These results indicate high tissue specificity of root ion flux responses to ROS and suggest that measuring the magnitude of H_2O_2-induced net K^+ and Ca^{2+} fluxes from mature root zone may be used as a tool for cell-based phenotyping in breeding programs aimed to improve salinity stress tolerance in cereals. In the follow-up work we have found a major QTL conferring ROS control of ion flux in roots that coincided with the major QTL for the overall salinity stress tolerance.

Conclusion:
These findings open prospects for improving salinity tolerance by targeting a previously unexplored trait and then pyramiding it alongside with more traditional Na^+ exclusion traits.

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EFFECTS OF SUPEROXIDE RADICALS ON MEMBRANE TRANSPORT ACTIVITY IN ROOT ANF LEAF MESOPHYLL TISSUES IN HALOPHYTES AND GLYCOPHYTES

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Salinity stress is a major environmental hurdle that restricts agricultural crop production across the globe. Amongst other factors, salinity stress-induced ROS production is considered to be one of the major constraints limiting plant growth and productivity in saline soils. Well before ROS can cause oxidative damage to plants, elevation in ROS levels cause a major disturbance to plant ionic homeostasis by activation of a broad range of Na^+, K^+ and Ca^{2+}-permeable channels and thus affecting cell metabolism. While most of the previous studies were focused of effects of H_2O_2 and hydroxyl radicals on activity of plant membrane transporters, their regulation by superoxide radicals ($O_2^{\cdot-}$) is studied much less. In this study we used methyl viologen (MV) as tool to probe the role of $O_2^{\cdot-}$ in regulating membrane-transport activity and systemic acquired resistance in two halophytic (*Chenopodium quinoa* and *Chenopodium album*) and one glycophytic (spinach) plants. In leaf mesophyll, application of MV resulted in a massive K^+ loss which was 3-fold stronger in spinach compared with both halophytic species. MV treatment also caused transient Ca^{2+} uptake in halophytes, while in spinach net Ca^{2+} efflux was measured. In roots, MV-induced K^+ efflux was also strongest in spinach. Contrary to previously published work with H_2O_2, no difference in sensitivity to $O_2^{\cdot-}$ was found between elongation and mature zones. Regardless of the species, MV induced massive Ca^{2+} efflux from root epidermis. Taken together, this data suggests that the patterns of ion flux responses to $O_2^{\cdot-}$ differ from those to H_2O_2 or hydroxyl radicals, and that Ca^{2+} efflux systems play a major role in shaping stress-specific Ca^{2+} signatures in leaf and root tissues.

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REACTIVE OXYGEN SPECIES PRODUCTION IN THE INITIATION OF LEAF SENESCENCE IN FIELD- AND LABORATORY-GROWN BARLEY

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Leaf senescence is important process for remobilization of nutrients and grain filling in plants, but the initiation mechanisms are still poorly understood. One of the most expected candidates triggering leaf senescence is the alteration of photosynthetic electron transport in the thylakoid membrane that has impacts on the redox level and reactive oxygen species (ROS) production in chloroplasts. However, the initiation of leaf senescence can be affected by various environmental variations, which makes it difficult to generalize the sequences of leaf senescence. Recently, the different senescence scenarios in chloroplasts have been shown in two barley varieties grown in field conditions: “Lomerit” shows a decrease in the activity of photosystem (PS) II prior to that of PSI and accumulates two type ROS, $^1O_2$ and O_2^-, and “Carina” that first loses PSI activity earlier and generates only O_2^- (Krieger-Liszkay et al. 2015 *Planta* 241, 1497–1508). Here, we investigated changes of photosynthetic electron transport and ROS production at the early stage of leaf senescence in these two barley varieties to identify the key factor for initiation of leaf senescence in two growth conditions (field and controlled-laboratory). ROS generation was analyzed using spin trapping EPR spectroscopy, and changes in photosynthetic electron transport were detected *in vivo* by absorption changes in the near infrared spectral region allowing to follow changes in P700 (the reaction center chlorophyll of PSI), plastocyanin, and ferredoxin. Loss of plastocyanin was observed at the early stage of leaf senescence by spectroscopic and immunological analyses. Finally, we discuss these findings in the context of the initiation of leaf senescence.

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ALLEVIATION OF GLYPHOSATE-INDUCED OXIDATIVE STRESS IN *SOLANUM LYCOPERSICUM* L. BY NITRIC OXIDE

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Glyphosate (GLY) is currently the most used herbicide worldwide, given its great efficacy and non-selective action towards weeds. Although GLY quickly degrades when in contact with soil, recent studies have been reporting non -target toxicity for important crops, such as tomato (*Solanum lycopersicum* L.) and barley (*Hordeum vulgare* L.). Thus, new strategies to minimize the effects of GLY residues on non-target plants are urgently needed to ensure food safety. In this context, nitric oxide (NO) can be a good candidate, since this gaseous molecule is recognized for its ability to improve plant tolerance to different abiotic stresses. Therefore, the present work aims at evaluating the potential of NO (200 µM sodium nitroprusside) to alleviate GLY (10 mg kg^{-1} soil) oxidative stress in tomato plants, particularly focusing on the cellular redox homeostasis. After 28 days, plant growth was severely repressed by GLY in both shoots and roots, being this negative effect partially reverted by the co-application of NO. In what regards the oxidative stress markers, results showed that lipid peroxidation and reactive oxygen species (ROS) levels were enhanced after exposure to GLY in shoots of tomato plants, but not in roots. Regarding the antioxidant performance, GLY induced the overaccumulation of proline and glutathione and upregulated the activity of superoxide dismutase (SOD), while not significantly changing the activity of ascorbate peroxidase and glutathione S-transferase, and the levels of ascorbate in shoots. Upon co-treatment with NO, GLY-induced oxidative stress was reduced, with lower levels of ROS, lipid peroxidation and ascorbate, and enhanced activities of SOD and GST on shoots; proline and glutathione contents did not change. Overall, our results point towards a protective effect of NO against GLY phytotoxicity in tomato plants, by limiting the occurrence of oxidative damage and stimulating detoxification of the herbicide.

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ALLELOPATHY INDUCED OXIDATIVE STRESS IN RADISH SEEDLINGS

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Allelopathy plays an important role in plant-plant competition. Some plants can release allelopathic compounds in the soil and therefore induce production of reactive oxygen species (ROS) in nearby plants. Consequently, the growth of nearby plants can be inhibited due to oxidative stress. Japanese knotweed (*Fallopia japonica*) and Bohemian knotweed (*F.* ×*bohemica*) are invasive alien plants in Europe and North America. They reduce species diversity by allelopathy. The aim of our study was to evaluate the effect of Japanese and Bohemian knotweed rhizome extracts on oxidative stress related morphological and biochemical characteristics in radish seedlings.

Seeds of radish (*Raphanus sativus*) were exposed to aquaeous extracts of knotweed rhizomes with concentrations of 0.5%, 1%, 2%, 5% and 10% (w/v). After 3, 5 and 7 days, the length of primary root, lateral roots and shoot were measured on digital photos with *ImageJ* 1.x software. Stress related biochemical parameters were analysed spectrophotometrically: total antioxidative capacity (TAC), lipid peroxidation measured via the content of malondialdehyde (MDA), activity of antioxidant enzymes catalase (CAT) and guaiacol peroxidase (G-POD). Accumulation of H_2O_2 in seedlings was localized with diaminobenzidne (DAB) and analysed with stereo microscope.

Our study showed that the main target of knotweed´s allelopathic compounds was the primary root. Extracts of both knotweeds inhibited the growth of primary root for more than 60%, whereas shoots were not affected. Their mode of action was concentration-dependent and had similar effect on the growth of lateral roots. Concentration of TAC increased in roots of exposed seedlings, which can be the reason that MDA remained mainly at the control level. Staining with DAB showed the increased synthesis of H_2O_2 in roots, especially in root tips.

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STEM CELL FATE IN HYPOXIC ROOT APICAL MERISTEMS IS INFLUENCED BY PHYTOGLOBIN EXPRESSION

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Root survival to flooding-induced hypoxic stress is dependent upon maintaining the functionality of the apical root meristem quiescent center (QC), a process that is governed by the PIN1-mediated auxin flow leading to the formation of an auxin maximum which is needed for the establishment of a highly oxidized environment specifying the QC niche. Perturbations in auxin flow and distribution along the root profile occurring during hypoxia can shift the redox state of the QC towards a more reduced environment leading to the activation of the QC, degradation of the meristem, and root abortion. The maize phytoglobin *ZmPgb1.1* is involved in minimizing these damaging effects during hypoxia in processes that result in sustaining the PIN-mediated auxin maximum and an oxidized environment in the QC. The oxidized environment is accomplished by maintaining the activity of redox enzymes oxidizing ascorbate and glutathione. These events, compromised in QCs suppressing *ZmPgb1.1*, ensure the functionality of the QC and root meristems under conditions of low oxygen resulting in stable root performance.

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DO CANAVANINE AND *META*-TYROSINE IMPACT RNA NITRATION IN ROOTS OF TOMATO SEEDLINGS?

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Canavanine (CAN) and *meta*-Tyrosine (*m*-Tyr) belong to non-protein amino acids (NPAAs). CAN, the structural analog of arginine is found predominantly in seeds of Fabaceae plants and acts in plant defense against herbivores. *m*-Tyr is a structural analogue of phenyloalanine and as a component of exudates of fescues species is considered as a strong allelopathic compound. ROS and RNS are cellular mediators in plant responses to various environmental stresses, including also allelopathic or phytotoxic interactions (Gniazdowska et al. 2015). Seedlings of tomato (*Solanum lycopersicum* L.) were treated for 72 hours with *m*-Tyr (50 and 250 µM) or CAN (10 and 50 µM), control plants were cultured in water. Inhibition of elongation growth of tomato roots by NPAAs was accompanied by induction of oxidative stress expressed as enhanced production of ROS ($O_2^{\bullet-}$ and H_2O_2). CAN inhibited NO emission, while did not influenced $ONOO^-$ content in the tissue. *m*-Tyr at lower concentration had no impact on NO or $ONOO^-$ content in tomato roots, while *m*-Tyr at higher concentration lowered NO emission and slightly increased $ONOO^-$ level. Modifications in ROS and RNS production level by NPAAs resulted in declined level of nitrated proteins (3-NT) in CAN treated plants, and elevated level of 3-NT after 250 µM *m*-Tyr stress. Besides ROS and RNS dependent post-translational modification of proteins, the more attention is focused on $ONOO^-$ induced nitration of guanine in DNA or/and RNA. Nitration of guanine results in formation of 8-nitro-guanine. Supplementation of tomato seedlings with CAN or *m*-Tyr decreased level of nitrated RNA in roots, and the decline in RNA nitration level was more drastic in *m*-Tyr exposed tissue.

References.

Gniazdowska A. et al. (2015). Allelopathic compounds as oxidative stress agents: Yes or NO. doi: 10.1007/978-3-319-10079-1_8.

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Posters

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ANALYSING THE ROLE OF THE *ARABIDOPSIS* PROTEIN KINASE ASKα IN OXIDATIVE STRESS

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Abiotic stress leads to accumulation of reactive oxygen species (ROS) in different cellular compartments. On the one hand ROS plays a key role in cell signalling; on the other hand excess levels of ROS are toxic for the cell. Previously, we have shown that the *Arabidopsis* protein kinase ASKα contributes salt stress tolerance and modulates part of the cellular redox balance. ASKα regulates the activity of glucose-6-dehydrogenase (G6PD) under salt stress conditions. G6PD is the key enzyme of the oxidative pentose phosphate pathway that generates reducing equivalents that can be used to detoxify excess levels of ROS under high salinity conditions. Interestingly, in addition to salt, ROS treatment is able to induce ASKα activity indicating a possible role of ASKα in ROS-triggered responses. Currently, we are investigating whether ASKα plays a role in the response of plants to oxidative stress and will present data on the response of Arabidopsis plants overexpressing or deficient in ASKα to ROS generated in different cellular compartments.

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BEYOND THE INHIBITION OF COPPER AMINE OXIDASE: L-AMINOGUANIDINE AND POLYAMINE CATABOLISM IN TOMATO PLANTS AFTER SALT STRESS

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Polyamines are essential N-containing polycations which function not just during the developmental growth responses but also in biotic and abiotic stress conditions in plants. The optimal polyamine homeostasis strongly depends on their catabolism by two enzymes, copper amine oxidase (CuAO) and polyamine oxidase (PAO). During these enzyme reactions, hydrogen peroxide and aldehydes or ammonia could be produced. In order to investigate the role of copper amine oxidase in tomato salt stress responses, we used L-aminoguanidine treatment as a pharmacological approach. L-aminoguanidine is an inhibitor compound with versatile functions in plants and animal organisms as well. Most of experiments used it as an iNOS inhibitor; however it can also inhibit the CuAO activity. We used tomato (*Solanum lycopersicum* Mill. L. cv. Rio Fuego) plants in hydroponic culture in the greenhouse. Salt stress was applied by 100 mM NaCl into the nutrient solution. We found that L-aminoguanidine had an organ-dependent effect on CuAO and PAO enzyme activities; however the level of hydrogen peroxide did not show any significant differences. The enzymatic and non-enzymatic antioxidant defence system were also analysed after L-aminoguanidine treatment in order to determine the oxidative stress generating or scavenging effect of L-aminoguanidine during salt stress. After analysing the free polyamine spectra by HPLC, we found that L-aminoguanidine was effective to modify the composition of free polyamines and this response could be affected by reduced nitric oxide level proved by microscopic staining. The main goal of this work is to enhance our knowledge about the multifunctional role of L-aminoguanidine and related responses of polyamine catabolism in plants.

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ROS TRACKING: REDOX COUPLING OF SUBCELLULAR COMPARTMENTS DURING PHOTO-OXIDATIVE STRESS TRIGGERED IN CHLOROPLASTS

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Metabolic changes in chloroplasts and mitochondria trigger retrograde signals to feedback information to nuclei. One proposed signal for this are reactive oxygen species (ROS), high concentrations of which may cause oxidation of protein thiol switches and also glutathione (GSH) to form glutathione disulfide (GSSG). An imbalance of the GSH/GSSG pool is considered an important marker for stress responses. Genetically encoded biosensors Grx1-roGFP2 and roGFP2-Orp1 enable *in vivo* visualization of the GSH redox potential and H2O2 production, respectively. At subcellular level, however, microscope-based approaches are limited by sample throughput and our ability to do long-term dynamic recordings. To overcome these constrains, we implemented a plate reader-based system that enables monitoring the GSH/GSSG dynamics and H2O2 production in a high-throughput manner in intact samples exposed to different types of stress over time spans of several hours. The use of compartment-targeted versions of Grx1-roGFP2 and roGFP2-Orp1 together with specific inhibitors of photosynthetic electron transport and the non-selective herbicide methyl viologen (MeV), reveal a more sensitive GSH pool in plastids compared to the cytosol upon MeV treatments. Similarly, MeV induces an autonomous oxidation in the mitochondrial matrix that is dependent on electron flux through the mitochondrial electron transport chain. In addition, we show that under illumination with photosynthetically active light the initial oxidation triggered by MeV in chloroplasts propagates into the cytosol and mitochondria where it results in an oxidation surpassing the mitochondria-autonomous oxidation. Based on this so far inaccessible spatiotemporal resolution, we discuss the subcellular compartment-specific oxidative responses in the context of retrograde signaling and general stress acclimation in plants.

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OXYGEN REDUCTION IN CHLOROPLASTS AND THE ROLE OF HYDROGEN PEROXIDE IN REGULATION OF THE PSII ANTENNA SIZE

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The electron flow to oxygen in chloroplasts leads to the formation of reactive oxygen species, such as superoxide radical and hydrogen peroxide (H_2O_2). We demonstrated that the H_2O_2 production occurred in both water phase, *i.e.* stroma, and lipid phase, *i.e.* thylakoid membrane. Further, we established that the membrane H_2O_2 formation is the result of interaction of fully reduced plastoquinone, PQH_2, with superoxide anion radical.

It is known, that one of the main responses of long-term acclimation to high light is the decrease in the light-harvesting antenna size of PSII that is regulated by the redox state of the PQ pool. The literature data provide evidence that the decrease in the antenna size occurs when the PQ pool is highly reduced that is favorable for H_2O_2 generation in the membrane. Therefore, using barley (*Hordeum vulgare*) plants, we investigated whether H_2O_2 is involved in regulation of the PSII antenna size under illumination.

The hydrogen peroxide content was measured based on the luminol peroxidative oxidation. The changes in the PSII antenna size were examined by electrophoretic techniques and PCR analysis. The PQ pool reduction level was assayed by the chlorophyll *a* fluorescence measurements. It was found that the reduction of the PSII antenna size was suppressed in HL in leaves characterized by the high reduction level of the PQ pool but the low hydrogen peroxide content. In opposite, a decrease in the antenna size was observed in low light in the presence of hydrogen peroxide at elevated concentration in leaves.

The data obtained in this work confirm that the H_2O_2 molecules represent the signal messengers by which the redox state of the PQ pool provides its regulatory effect under changes in environmental light conditions.

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MINING FOR ROS-SENSORS IN PLANTS: SITE IDENTIFICATION OF SULFENYLATED CYSTEINES *IN VIVO*

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Hydrogen peroxide (H_2O_2) is an important messenger molecule for diverse cellular processes. H_2O_2 oxidizes proteinaceous cysteinyl thiols to sulfenic acid, also known as S-sulfenylation, thereby affecting the protein conformation and functionality. Although many proteins have been identified as S-sulfenylation targets in plants, site-specific mapping and quantification remained largely unexplored, certainly in a comprehensive manner. Therefore, we implemented two new proteomic workflows to directly identify the oxidized cysteines residues within *Arabidopsis thaliana* cell culture sense and response to H_2O_2 stress.

a) Before, by using a genetically encoded probe (YAP1C-GS), we identified approximately 100 nuclear and cytosolic proteins as being sulfenylated. However, the identity of the S-sulfenylated cysteines remained unknown. Therefore, we designed a new approach implementing an extra immunopurification step based on a seven-amino-acid YAP1C derived peptide (CSEIWDR), which is exposed after trypsin digestion. By this, we identified approximately 800 S-sulfenylated sites in 557 proteins in *Arabidopsis thaliana cells* upon H_2O_2 treatment.

b) We implemented a benzothiazine-based chemical probe (BTD), which consists of a dimedone scaffold for S-sulfenylated cysteines recognition and an alkyne handle for protein enrichment, within a quantitative chemoproteomic platform. Thereby, we performed a comprehensive quantitative and site-specifically profile of *in vivo* protein S-sulfenylation in *Arabidopsis thaliana* cells. Using this optimized quantitative workflow, we mapped 1,537 S-sulfenylated sites on more than 1,000 proteins in *Arabidopsis thaliana* cells.

Together, two new proteomic strategies enabled a comprehensive assessment of redox-sensitive cysteines in *Arabidopsis thaliana* under H_2O_2 stress, and deliver an unprecedented platform for our further understanding of redox signaling in plants.

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FUNCTIONAL ANALYSIS OF *A. THALIANA* SELENOPROTEIN H HOMOLOGS

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Selenium is an essential micronutrient for many organisms, with concentration dependent beneficial and toxic effects, respectively. It is required for biosynthesis of selenocysteine (Sec, U), the twenty first naturally occurring amino acid encoded by the UGA codon. Eukaryotic selenoproteomes are highly variable in size, with vertebrates and algae having big sets of these proteins while other organisms, such as higher plants and fungi, have lost all selenoproteins during the course of evolution. The human genome encodes for 25 selenoproteins of which the best described are glutathione peroxidases, thioredoxin reductases and thyroid hormone deiodinases. Recent studies have led to a significant progress in the functional analysis of several other selenoproteins, such as MsrB, Selenoprotein P (SelP), Selenoprotein N and SPS2. Recently, the function of Selenoprotein H (SelH) during development, oxidative stress responses and glutathione biosynthesis has been established. SelH possesses a highly conserved CXXU motif which strongly suggests a redox-related function. Through a genome mining approach, we identified two closely related SelH homologs in *Arabidopsis thaliana*. Both have a conserved thioredoxin-like CXXC motif. AtSelH homologs share a unique subcellular localisation, but have distinct spatio-temporal expression characteristics. Overexpression of *AtSelHs* lead to profound morphological and developmental perturbations whereas single and double knockout plants do not exhibit any visible phenotypes. Functional analysis of both proteins will be discussed.

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INSIGHT INTO LAND PLANT EVOLUTION FORM A LIVERWORT PERSPECTIVE: DOES REDOX MATTER?

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Land plants evolved over 500 MYA from an ancestral charophycean alga and had a major impact in transforming our terrestrial environment. The bryophyte *Marchantia polymorpha* exhibits several features that make it an ideal basal land plant model organism that is currently intensively exploited to understand the evolution of novel developmental, biochemical and cellular attributes mediating the adaptation to a life on land. This liverwort has not undergone paleoploidisation events and exhibits a low genetic redundancy of transcription factor activity, which often hinders functional studies in higher land plants. Recent Marchantia genome analysis revealed the presence of only one single TCP-P and TCP-C gene, bZIP transcription factors controlling cell proliferation and also diverse other processes in angiosperms. The establishment of a molecular toolbox comprising efficient Marchantia genome editing and transformation methods was exploited to generate and further analyze knockout Mp*tcp1* mutants. The Mp*TCP1* function in the control of developmental as well as redox-processes will be discussed and we are establishing redox-sensors to monitor redox-regulation in this liverwort.

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ARABIDOPSIS XDO1 ENCODES A PHOSPHOLIPASE C-LIKE PROTEIN INVOLVED IN MODULATING ROS AND SALICYLIC ACID RELATED DEFENSE RESPONSE IN A LIGHT-DEPENDENT MANNER

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AT4G34930 encodes a phosphatidylinositol-specific phospholipase C (PLC) X domain containing protein named XDO1 (X DOMAIN CONTAINING PROTEIN1). It was found to be correlated with genes that are responsive to biotic stimuli. To explore a potential role in plant defense, loss-of-function alleles were isolated and characterized. The *xdo1* mutant exhibits a light-dependent lesion mimic phenotype, which is not dependent on day length or light intensity per se, but rather on the total light impact in terms of intensity and duration that plants have received. In addition, the lesion mimic phenotype is accompanied by enhanced ROS and salicylic acid (SA) levels. Marker genes of SA biosynthetic and the SA signaling pathway are upregulated. When a mutation of the key SA biosynthetic gene *ISOCHORISMATE SYNTHASE1 (ICS1)* was introduced into *xdo1* mutant, the light-dependent lesion mimic phenotype was abolished. Furthermore, the *xdo1* mutant is more resistant to infection with *Pseudomonas syringae* pv. *tomato* DC3000. A non-targeted metabolomic analysis using FT-ICR MS indicated changes in some very long chain fatty acids in the *xdo1* mutant in comparison to wild type. Taken together, these results suggest that XDO1 may play a role in regulating lipid synthesis or homeostasis and thereby modulates ROS and SA-related defense response.

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REGULATION BY NITRIC OXIDE ON SPHINGOLIPID METABOLISM OF PEACHES DURING STORAGE

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This study was conducted to investigate the roles of nitric oxide (NO) and cold signal in regulating the responses of sphingolipids metabolism to chilling injury of peach fruit during storage. Peaches were treated by immersion in distilled water (control) and 15 μmol L^{-1} NO solution, then stored at 25 °C and 0 °C, respectively. The changes of the enzymes activities of sphingolipid metabolism and the contents of sphingolipid in peach fruits during storage were studied. The results showed that NO significantly increased the contents of sphingolipids and the activities of key enzymes including phospholipase A (PLA), phospholipase C (PLC), phospholipase D (PLD), 3-ketodihydrosphingosine reductase (3KSR), sphingosine kinase (SPHK), ceramide synthase (CERS) and ceramide kinase (CERK) in sphingolipid metabolism in peaches during storage. On the contrary, cold storage can reduce the activities of these enzymes. The activities of acid phosphatase (AP) and alkaline phosphatase (ALP) in NO treated peaches maintain higher levels at 25 °C, while NO could reduce the activities of these enzymes at cold-stored to adapt stress. These results suggested that exogenous NO and cold signaling were antagonistic to maintain sphingolipid metabolism. NO could maintain the synthesis of sphingolipids by maintaining the activities of key enzymes in sphingolipid metabolism, thereby sustain proper contents of sphingolipids, enhance the stability of cell membrane and delay the decrease of fruit quality.

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REGULATION OF MITOCHONDRIAL RESPIRATORY PATHWAYS DURING STRESS CONDITIONS

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Plant mitochondria have essential role in regulation of numerous metabolic pathways that are important in adaptive responses to unfavorable conditions. During stress adaptation the stabilization of the electron flow in the mitochondrial electron transport chain (ETC) can protect plants by reduction of oxidative damage, control of redox balance and support of photosynthesis. Reactive oxygen species (ROS) can be produced in the mitochondrial ETC under stress, where Complex I and III are the major sites of ROS synthesis. Thereby the mutations in genes encoding the mitochondrial proteins might due to changes in stress responses. We have started the detailed characterization of numerous Arabidopsis thaliana mutants, in which the mutations localized in the subunits of Complex I and III. We worked forth with 13 lines started the primary screening to search for plants, that showed morphological and physiological changes under stress conditions compared to the wild type. We performed in vitro germination and growth tests, and in collaboration with European Plant Phenotyping Network the drought and salt tolerance of the mutants were examined also. Several interesting mutants were found and one line was chosen, which showed tolerance to drought stress. The localization of the mutation is in a gene what encodes the NDUFS8.2 protein. The *ndusf8.2-1* mutant was also tolerant to oxidative and osmotic stresses: less hydrogen peroxide formed in them and lipid peroxidation level was lower compared to the wild type plants. Changes in chlorophyll fluorescence under stress treatments suggested that these Complex I mutations influence photosynthesis as well. Our data shows that the *NDUSF8.2* gene has important affect on plants stress responses, and we found a strong correlation between the mutations and the photosynthetic activity and energy production.