Abstract book and Information for the 13th International Sulfur Workshop

September 21 -25, 2025
Heidelberg, Germany

Content list

Scientific Program
13th International Sulfur Workshop

Sunday 21st September

14.00 – 17.00	**Registration (Lecture Hall INF 230)**
17.00 – 20.00	**Get-together (Drinks and Snacks included)**

Monday 22nd September

09:00 – 09.15	**Opening remarks**
09.15 – 14.00	**Session 1: Sulfur metabolism**
09.15 – 10.00	**Keynote lecture: Barbara Halkier (University of Copenhagen, Denmark)** Pathway and transport engineering of glucosinolates
10.00 – 10.30	**Masami Hirai (RIKEN Center, Yokohama, Japan)** Metabolomics-based insights into glucosinolate metabolism and function in plant adaptation
10.30 – 11.00	**Coffee break**
11.00 – 11:20	**Yael Hacham (Tel-Hai Academic College, Israel)** Abiotic-Stress Control of Methionine Flux: CGS Destabilization and MGL-Driven Isoleucine Production
11.20 – 11.40	**Frédéric Marsolais (University of Western Ontario, Canada)** Inhibition of β-substituted alanine synthase 4;1 from common bean (*Phaseolus vulgaris* L.) by benzoic and salicylic acids
11.40 – 12.00	**Tatjana M. Hildebrandt (University of Cologne, Germany)** Thermal proteome profiling identifies mitochondrial aminotransferases involved in cysteine catabolism via persulfides in plants
12.00 – 12.20	**Stephan Wagner (University of Bonn, Germany)** A balancing act: the role of lipoyl synthase in plant mitochondrial metabolism and sulfide toxicity
12.20 – 14.00	**Lunch**

14.00 – 16.40	**Session 2: Redox & Glutathione metabolism**
14.00 – 14.45	**Keynote lecture: Andreas Meyer (University of Bonn, Germany)** Glutathione metabolism and its implications for growth and redox signalling
14.45 – 15.15	**Markus Schwarzländer (University of Münster, Germany)** A redox metabolic twist to plant immunity signaling
15.15 – 15.35	**Frank van Breusegem (VIB-UGENT, Belgium)** Mapping the Sulfenome: Redox Proteomics Reveal Functional Cysteine Oxidation in Plant Stress Response
15.35 – 16.00	**Coffee break**
16.00 – 16.20	**Naoko Ohkama-Ohtsu (Tokyo University of Agriculture & Technology, Japan)** Roles of glutathione degradation in sulfur metabolism in plants
16.20 – 16.40	**Akiko Maruyama-Nakashita (Kyushu University, Japan)** Contribution of sulfate transporter SULTR2;1 in plant transition from vegetative to reproductive growth through long-distance sulfate transport
16.40 – 17.00	**Break**
17.00 – 18.30	**Poster session with Snacks** (Odd Numbers)
Evening	**Meet your Friends at selected Restaurants in Heidelberg.**

Tuesday 23rd September

09.15 – 14.00	**Session 3: Interactions with Other Nutrients**
09.15 – 10.00	**Keynote lecture: Fang-Jie Zhao (Nanjing Agricultural University, China)** Interactions of sulfur nutrition with essential micronutrients and toxic metals/metalloids
10.00 – 10.30	**Hatem Rouached (Michigan State University, USA)** Ascorbate-Driven Chloroplastic Signaling Integrates Iron, Phosphorus, and Sulfur Deficiency to Sustain Photosynthesis
10.30 – 11.00	**Coffee break**
11.00 – 11:15	**Ikue Yamada (Hokkaido University, Japan)** Sulfur regulates the expression of phosphorus starvation responsive genes in rice
11.15 – 11.35	**Eleonora Coppa (University of Tuscia, Italy)** Sulfur-iron interplay in durum wheat: metabolic responses and food safety
11.35 – 11.55	**Ann Cuypers (Hasselt University, Belgium)** Cadmium stress intensity activates differential ER stress outcomes in *Arabidopsis thaliana*
11.55 – 12.15	**Andreu Cera (University of Caen-Normandy, France)** Sulfur accumulation as the physiological driver of gypsum specialization
12.15 – 12.30	**Muna A. Abdalla (Kiel University, Germany)** Metabolic reprogramming by sulfur in the presence of selenium
12.30 – 14.00	**Lunch**

14.00 – 16.30	**Session 4: Sulfur Signaling**
14.00 – 14.30	**Cecilia Gotor (University of Seville, Spain)** Hydrogen Sulfide: An Ally in Plant Survival and Stress Resilience
14.30 – 15.00	**Agnieszka Sirko (Polish Academy of Sciences, Poland)** Novel functions of LSU proteins
15.00 – 15.20	**Rachel Amir (Migal, Galilee Technology Center, Israel)** Elevated Methionine Alters Sulfur Metabolism and Epigenetic Landscapes in Arabidopsis
15.20 – 15.50	**Coffee break**

	Xin-Yuan Huang (Nanjing Agricultural University, China)
15.50– 16.10	The GTE7–IES2B-GTE2 complex epigenetically regulates sulfur homeostasis in *Arabidopsis thaliana*
	Hideki Takahashi (Michigan State University, USA)
16.10 – 16.30	Sulfur-JA signaling interactions manifesting metabolic tradeoffs in Arabidopsis
16.40 – 17.00	**Break**
17.00 – 18.30	**Poster session with Snacks** (even numbers)
Evening	**Meet your Friends at selected Restaurants in Heidelberg.**

Wednesday 24th September

09.15 – 14.00	**Session 5: Environmental Interactions**
09.15 – 09.45	**Stanislav Kopriva (University of Cologne, Germany)** Sulfur metabolites in plant microbe interactions
09.45 – 10.15	**David Mendoza Cozatl (University of Missouri, USA)** Unraveling the molecular mechanisms mediating the crosstalk between iron and sulfur homeostasis in rice
10.15 – 10.35	**Sophie Hendrix (Hasselt University, Belgium)** Double trouble: combined heat stress and iron toxicity synergistically affect cellular redox homeostasis in *Arabidopsis thaliana*
10.35 – 11:05	**Coffee break**
11.05 – 11.25	**Suyan Yee (The Australian National University, Australia)** Unravelling cellular communication networks and antioxidant metabolism driving stress-resilient C4 photosynthesis at cell-type resolution
11.25 – 11.40	**Büsra Elkatmis (University of Cologne, Germany)** Investigating the role of sulfur metabolism in the beneficial interaction between Pseudomonas argentinensis SA190 and Arabidopsis under drought
11.40 – 11.55	**Pratikshya Joshi (Université Bourgogne Europe, France)** How does sulfur nutrition affect the morphology and functioning of the nodulated root system of pea plants under water deficit?
11.55 – 12.15	**Sheng-Kai Sun (Heidelberg University, Germany)** The plastid cysteine synthase complex regulates ABA biosynthesis and stomatal closure in Arabidopsis
12.15 – 12.35	**Dimitris Bouranis (Agricultural University of Athens, Greece)** Boosting crop physiology – Alternative S-containing phytoboosters and systemic resistance inducers

12.35 – 14.00	**Lunch**

14.00 – 17.30	**Session 6: Applied Sulfur Technologies**
14.00 – 14.45	**Keynote lecture: Eve-Lyn S. Hinckley (University of Colorado, USA)** Advancing Investigation of Sulfur from the Molecular to the Global Scales
14.45 – 15.15	**G.-H. Crystal Ng (University of Minnesota, USA)** "Cryptic" Sulfur Cycling in Hydrologically Dynamic, Vegetated Wetlands
15.15 – 15.35	**Daniele Visioni (Cornell University, USA)** Using stratospheric sulfate aerosols to reduce climate change impacts: a climate and environmental science perspective
15.35 – 16.00	**Coffe break**
16.00 – 16.20	**Christian Zörb (University of Hohenheim, Germany)** Sulfur application in arable crops - recent approaches
16.20 – 16.40	**Pinnapat Pinsorn (Chulalongkorn University, Thailand)** The role of sulfur metabolism in shaping durian flavor during fruit ripening
16:40 – 17.00	**Laura Wayne (CORTEVA Agriscience, USA)** Enhancing seed composition to feed and fuel the world
17.00 – 17.30	**General Discussion, Award Show, and Closing Ceremony**
17.30 – 18.00	**Transfer to Wineyard Clauer**
18.00 – 19.00	**Winetasting at Wineyard Clauer**
19.00 – open	**Conference Dinner**

Thursday 25th September

Optional Excursions

9.30 Departure from Marktplatz/Herkulesbrunnen for Heidelberg Castle visit

14.15 Departure from Neckarstaden 25 for the 4-castle round trip (via River Neckar)

Abstracts of Oral Presentations

Session 1 Sulfur Metabolism

S1-1 Pathway and transport engineering of glucosinolates

Barbara Ann Halkier

DynaMo Center, Copenhagen Plant Science Center, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Glucosinolates are defense compounds characteristic of the Brassicales order, including the oilseed rape, broccoli and the model plant *Arabidopsis*. We use glucosinolates in Arabidopsis as a model system to study transport processes from site of biosynthesis to site of storage. Unravelling the transporter complement of glucosinolates will advance our understanding of the transport processes that move glucosinolates intracellularly and between cells at the tissue and whole organismal level. We have generated molecular tools to apply pathway and transport engineering of glucosinolates to increase availability of plant-based protein for human consumption, to improve human health and to increase resistance of brassicaceous crops. Our progress along those lines will be presented.

S1-2 Metabolomics-based insights into glucosinolate metabolism and function in plant adaptation

Masami Y. Hirai[1,2]

[1]*Metabolic Systems Research Team, RIKEN Center for Sustainable Resource Science, Yokohama, Japan*

[2]*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan*

Plants, as sessile organisms, must finely balance growth and environmental adaptation under often limited nutrient conditions. As a result, plant metabolism is intricately and tightly regulated, and individual metabolites are increasingly recognized as possessing a broader range of physiological functions than previously appreciated.

In my research, I have employed metabolomics-based approaches to elucidate the regulatory mechanisms underlying plant metabolism and to uncover the physiological roles of plant metabolites. Our previous study demonstrated, at the molecular level, that glucosinolates—known for their role in biotic interactions—can also serve as a sulfur source, with their degradation acting as the trigger. More recently, we discovered that the breakdown of indole glucosinolates contributes to compensated cell enlargement—an increase in cell size in response to a reduction in leaf cell number.

In this presentation, I will highlight these findings, with particular emphasis on the metabolomics techniques that supported our discoveries.

References:

Sugiyama R, Li R, Kuwahara A, Nakabayashi R, Sotta N, Mori T, Ito T, Ohkama-Ohtsu N, Fujiwara T, Saito K, Nakano RT, Bednarek P and Hirai MY (2021) Retrograde sulfur flow from glucosinolates to cysteine in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U.S.A. **118**: e2017890118

S1-3 Abiotic-Stress Control of Methionine Flux: CGS Destabilization and MGL-Driven Isoleucine Production

Yael Hacham[1,2] and Rachel Amir[1,2]

[1]*Department of Biotechnology, Tel-Hai Academic College, Upper Galilee, Israel*

[2]*Plant sciences, MIGAL - Galilee Research Institute, Kiryat Shmona, Israel*

Abstract

Transgenic tobacco plants with higher levels of methionine show increased sensitivity to oxidative stress compared to wild-type plants. During oxidative stress, the synthesis of cysteine and glutathione increases rapidly, while the biosynthesis of methionine decreases [1]. Our research indicates that oxidized glutathione (GSSG) directly binds to cystathionine-γ-synthase (CGS), a key enzyme in methionine synthesis, and triggers its destabilization. Reducing the levels of CGS shifts the flux of cysteine from methionine production toward glutathione synthesis, which is essential for coping with oxidative stress [2]. Additionally, the relationship between methionine and abiotic stresses has been observed in Arabidopsis seeds exposed to heat or osmotic stress. In these conditions, methionine-γ-lyase (MGL), the enzyme responsible for methionine catabolism, is upregulated. MGL cleaves methionine into α-ketobutyrate, ammonia, and methanethiol. The catabolic product, α-ketobutyrate, is then channeled into isoleucine (Ile) biosynthesis, resulting in increased levels of isoleucine. Accumulated isoleucine can function as an osmoprotectant and an alternative respiratory substrate, supporting mitochondrial electron flow and ATP production when carbohydrate supplies are limited. Thus, elevated isoleucine levels help maintain the energy state of the seeds under stress [3]. Seeds with reduced MGL activity accumulate high levels of methionine but demonstrate lower germination efficiency under stress, highlighting the importance of methionine catabolism for energy homeostasis.

References:

1. **Hacham Y, Matityahu I, Amir R.** (2017) Transgenic tobacco plants having a higher level of methionine are more sensitive to oxidative stress. Physiol Plant. 160(3):242-252
2. **Hacham Y, Kaplan A, Cohen E, Gal M, Amir R.** (2024) Sulfur metabolism under stress: Oxidized glutathione inhibits methionine biosynthesis by destabilizing the enzyme cystathionine γ-synthase. JIPB 67(2) 87-100
3. **Hacham Y, Shitrit O, Nisimi O, Friebach M, Amir R.** (2023) Elucidating the importance of the catabolic enzyme, methionine-gamma-lyase, in stresses during Arabidopsis seed development and germination. Front Plant Sci. 14:1143021

S1-4 Inhibition of β-substituted alanine synthase 4;1 from common bean (*Phaseolus vulgaris* L.) by benzoic and salicylic acids

Zixuan Lu[1,2], Wojciech Witek[3], Milosz Ruszkowski[3], Barbara Imiolczyk[3], Nataliya Paulish[3], Jaya Joshi[1,2,4], Mariusz Jaskolski[3,5], Frédéric Marsolais[1,2]

[1]*Agriculture and Agri-Food Canada, London Research and Development Centre, London, Ontario, Canada*

[2]*Department of Biology, University of Western Ontario, London, Ontario, Canada*

[3]*Department of Structural Biology of Eukaryotes, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland*

[4]*Department of Wood Science, University of British Colombia, Vancouver, British Colombia, Canada*

[5]*Department of Crystallography, Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland*

The nutritionally essential sulfur amino acids, methionine and cysteine, are present at suboptimal levels in legumes, such as common bean (*Phaseolus vulgaris* L.). β-Substituted alanine synthase 4;1 (BSAS4;1) is the major isoform of cytosolic cysteine synthase present in developing seeds of common bean. There is evidence that in addition to cysteine, this enzyme is also involved in the biosynthesis of the non-protein amino acid *S*-methylcysteine, which accumulates in the form of a γ-glutamyl dipeptide. Here, we report the high-resolution structure of recombinant BSAS4;1. Unexpectedly, the crystal structure showed the presence of a molecule of benzoic acid near the active site, which appeared to be co-purified from *E. coli*. Kinetic analysis indicated that benzoic acid acts as a competitive inhibitor of BSAS4;1 with respect to *O*-acetylserine. A more detailed analysis revealed the presence of multisite inhibition interactions, with possibly four independent binding sites. The apparent K_i value for benzoic acid was equal to 50 µM, and IC_{50} values for benzoic acid and the structurally related salicylic acid were equal to 1 mM and 0.7 mM, respectively. Using developing cotyledons grown *in vitro*, quantification of incorporation of $^{13}C_3$- and ^{15}N-labeled serine into cysteine and downstream metabolites indicated that benzoic acid effectively inhibited cysteine biosynthesis *in vivo* at a concentration of 1.2 mM. The results of experiments tracking the incorporation of ^{13}C-labeled sodium thiomethoxide provided further evidence that BSAS4;1 may be involved in the formation of free *S*-methylcysteine, through the condensation of *O*-acetylserine with methanethiol.

S1-5 Thermal proteome profiling identifies mitochondrial aminotransferases involved in cysteine catabolism via persulfides in plants

Björn Heinemann[1], Jannis Moormann[1], Shivsam Bady[1], Cecile Angermann[1], Andrea Schrader[2] and Tatjana M. Hildebrandt[1]

[1]*Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Germany*
[2]*Data Science and Management, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Germany*

Cysteine is a central metabolite in plant sulfur metabolism, with key roles in biosynthesis, redox regulation, and stress responses. While a mitochondrial cysteine degradation pathway has been described, the enzyme catalyzing its initial transamination step remained unidentified (Höfler et al. 2016). We applied thermal proteome profiling (TPP) to Arabidopsis mitochondria to uncover cysteine-interacting proteins (Heinemann et al. 2025). TPP successfully detected known cysteine-utilizing enzymes, validating its utility in plant metabolic research. Among the newly identified proteins with cysteine-dependent thermal shifts were two aminotransferases previously annotated as alanine and aspartate aminotransferases that were able to catalyze the conversion of cysteine to 3-mercaptopyruvate *in vitro*. Together with the sulfurtransferase STR1 and the persulfide dioxygenase ETHE1, these enzymes complete the mitochondrial pathway for cysteine catabolism. Kinetic analysis suggests that alanine aminotransferase in particular may act *in vivo* at physiological cysteine concentrations. We also found that GABA aminotransferase activity is inhibited by cysteine, which may reflect a role in stress-related regulation. Beyond identifying candidate enzymes, the data set will be useful for investigating cysteine-mediated modulation of transporters, RNA-editing proteins, and respiratory chain components. Given cysteine's emerging role as a metabolic signal in stress responses, and the importance of allosteric regulation in amino acid metabolism, these findings highlight the broader regulatory potential of cysteine-protein interactions in plants.

References:

Höfler S, Lorenz C, Busch T, Brinkkötter M, Tohge T, Fernie AR, Braun HP, Hildebrandt TM (2016) Dealing with the sulfur part of cysteine: four enzymatic steps degrade l-cysteine to pyruvate and thiosulfate in Arabidopsis mitochondria. Physiologia Plantarum, **157(3)**: 352–366.

Heinemann B, Moormann J, Bady S, Angermann C, Schrader A Hildebrandt, TM (2025) Thermal proteome profiling identifies mitochondrial aminotransferases involved in cysteine catabolism via persulfides in plants. BioRxiv https://doi.org/10.1101/2025.05.23.655777.

S1-6 A balancing act: the role of lipoyl synthase in plant mitochondrial metabolism and sulfide toxicity

Stephan Wagner[1], Luca Pedroletti[1], Gernot Poschet[2], Maria Homagk[1], Tatjana Hildebrandt[3], Markus Wirtz[2], Rüdiger Hell[2] and Andreas J. Meyer[1]

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[2]*Heidelberg University, Centre for Organismal Studies, Heidelberg, Germany*

[3]*University of Cologne, Institute for Plant Sciences, Cologne, Germany*

Lipoyl synthase 1 (LIP1) is an evolutionarily conserved plant mitochondrial protein that catalyses protein lipoylation, an essential post-translational modification, which requires sulfur. LIP1 binds two iron-sulfur (4Fe-4S) clusters: a catalytic cluster, which remains intact during catalysis, and an auxiliary cluster which is dissembled to abstract the sulfur atoms required for the synthesis of the lipoyl co-factor.

Genetic restriction of the mitochondrial 4Fe-4S cluster assembly in the model plant *Arabidopsis thaliana* results in lipoylation defects and severely restricts plant growth, highlighting the importance of sustained LIP1 activity for plant mitochondrial metabolism (Moseler et al., 2021). On the other hand, constitutive overexpression of *LIP1* and increased abundance of LIP1 in Arabidopsis also restricts plant growth and we provide evidence that this is due to sulfide being released from the auxiliary 4Fe-4S cluster (Pedroletti et al., 2023).

We present and discuss the consequences of excessive sulfide release through constitutive *LIP1* expression and how *LIP1* overexpressors can be used experimentally to study assembly and transfer of mitochondrial 4Fe-4S clusters and the effects of excessive sulfide released in plant mitochondria.

References:

Moseler, A., Kruse, I., Maclean, A. E., Pedroletti, L., Franceschetti, M., Wagner, S.,. & Meyer, A. J. (2021). The function of glutaredoxin GRXS15 is required for lipoyl-dependent dehydrogenases in mitochondria. *Plant Physiology*, *186*(3), 1507-1525.

Pedroletti, L., Moseler, A., Timm, S., Poschet, G., Homagk, M., The, J. X.,. & Meyer, A. J. (2023). Altered iron-sulfur cluster transfer in Arabidopsis mitochondria reveals lipoyl synthase as a Janus-faced enzyme that generates toxic sulfide. *bioRxiv*, 2023-08.

Session 2 Redox and Glutathione Metabolism

S2-1 Glutathione metabolism and its implications for growth and redox signalling

Andreas Meyer[1]

[1]*Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany*

Glutathione was one of the first metabolites that cells developed against the destructive power of reactive oxygen, which increased dramatically during the Great Oxygenation Event approximately 2.4 billion years ago. In addition to its role in detoxifying reactive oxygen species, glutathione now has a wide range of other functions. These include redox controlling and signalling redox reactions, detoxifying electrophilic compounds, acting as a cofactor in metabolic reactions, and providing a sulfur donor for synthesising sulfur-containing metabolites. The developmental arrest observed in glutathione-deficient Arabidopsis mutants suggests a potential connection between glutathione and meristematic activity (Vernoux et al., 2000; Lee et al., 2025). However, our understanding of the impact of glutathione metabolism on growth remains limited.

It is generally assumed that glutathione-dependent redox signalling is mediated by type I glutaredoxins (GRXs). To further explore these signalling mechanisms, it is important to understand the subcellular localization of GRXs, their kinetic properties, and also the dynamics of the respective redox changes in terms of both time and space. Over the past two decades, the use of genetically encoded probes for H_2O_2 and the glutathione redox potential (E_{GSH}) has provided access to the respective physiological parameters in live cells (Wagner and Meyer, 2025). In combination with genetic approaches, they allow the genetic dissection of production and transmission systems in vivo. The talk will address various glutathione-dependent processes, focussing particularly on those mediated by GRXs, and will discuss our current understanding of how glutathione affects growth in Arabidopsis.

References:

Lee LR, Guillotin B , Rahni R, Hutchison C, Desvoyes B, Gutierrez C, Birnbaum KD (2025) Glutathione accelerates the cell cycle and cellular reprogramming in plant regeneration. Developmental Cell **60(8):** 1153-1167

Vernoux T,Wilson RC, Seeley KA, Reichheld J-P, Muroy S, Brown S, Maughan SC, Cobbett CS, Van Montagu M, Inzé D, May MJ, Sung ZR (2000) The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. The Plant Cell **12:** 97–109

Wagner S, Meyer AJ (2025) Illuminating plant metabolism with genetically encoded biosensors. Journal of Plant Physiology **311:** 154498

S2-2 A redox metabolic twist to plant immunity signaling

Pedro Barreto[1], Jan-Ole Niemeier[1], Elias Feitosa-Araujo[1], Daniela Döben[1], Lena Knorr[1], Lars Voll[2], Markus Schwarzländer[1]

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[2] *Molecular Plant Physiology, Department of Biology, University of Marburg, Marburg, Germany*

Redox metabolism in cells requires dynamic adjustment to constantly meet demands, maintain efficiency and avoid dysfunction. This is particularly relevant in plant cells which are directly exposed to frequent and often dramatic changes in their immediate environment, including physiological transitions and stressors. Yet, our understanding of the dynamics and the interaction between redox-metabolism and -signaling at the cell compartment level is limited. In order to shed light on those hidden dynamics we have been using quantitative confocal microscopy and fluorimetry to assess transitions in cell physiology *in vivo* using a growing set of genetically-encoded fluorescent protein-based redox sensors.

In this talk I will introduce our ongoing efforts to dissect a novel redox metabolic facet of the pattern triggered immunity (PTI) response. Highlighting intracellular NAD, NADP and glutathione redox dynamics in conjunction with H_2O_2 signatures, I will propose an unexpected link between immunity signaling and central metabolism.

S2-3 Mapping the Sulfenome: Redox Proteomics Reveal Functional Cysteine Oxidation in Plant Stress Response

Zeya Chen[1,2], Barbara De Smet[1,2] , Zhicheng Zhang[1,2], Didier Vertommen[3], Joris Messens[4,5], Pavel I Kerchev[1,2,6], Jingjing Huang[1,2] and Frank Van Breusegem[1,2]

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[5]*Structural Biology Research Center, VIB, 1050 Brussels, Belgium.*
[6] *Mendel University in Brno, 613 00 Brno, Czech Republic*

In aerobic life forms, reactive oxygen species (ROS) are produced by the partial reduction of oxygen during energy-generating metabolic processes. In plants, ROS production increases during periods of both abiotic and biotic stress, severely overloading the antioxidant systems. Hydrogen peroxide (H2O2) plays a central role in cellular redox homeostasis and signaling by oxidizing crucial cysteines to sulfenic acid, which is considered a biologically relevant post-translational modification (PTM). Over the past years we implemented several redox proteomics strategies to chart those proteins in which particular cysteines undergo at least the first oxidation step from free thiol to sulfenic acid (Willems et al., 2024). One approach is to express a synthetic version of the Yeast Activation Protein-1 (YAP1) in plant cells, at subcellular resolution, as a trap for S-sulfenylated proteins (De Smet et al., 2019). Here, we report on a nuclear variant of YAP1, identified 225 potential redox-active proteins undergoing S-sulfenylation. We confirmed the functional significance of S-sulfenylation of particular Cys-residues on the histone acetyltransferase GCN5 and demonstrate that Cys oxidation regulates the RNA-binding activity of class II polyA Binding Proteins (De Smet et al., 2025).

References:

De Smet B., Yang X., Plskova Z., Castell C., Fernández-Fernández A., Dard A., Masood J., Mhamdi A., Huang J., Vertommen D., Chan K.X., Pyr dit Ruys S., Messens J., Kerchev P.I.* and Van Breusegem F.* (2025). The nuclear sulfenome of Arabidopsis: spotlight on histone acetyltransferase GCN5 regulation through functional thiols. *J. Exp. Bot.* 76, 1569-1584.

Willems P.*°, Sterck L.°, Dard A., Huang J., De Smet I., Gevaert K. and Van Breusegem F.* (2024). The Plant PTM Viewer 2.0: in-depth exploration of plant protein modification landscapes. *J. Exp. Bot.* 75, 4611-4624.

De Smet B., Willems P., Fernandez-Fernandez A.D., Alseekh S., Fernie A.R., Messens J.* and Van Breusegem F.* (2019). *In vivo* detection of protein cysteine sulfenylation in plastids. *Plant J.* 97, 765-778.

S2-4 Roles of glutathione degradation in sulfur metabolism in plants

Takehiro Ito[1], Ryosuke Sugiyama[2], Hiroki Harada[3], Haruna Aoyama[3], Masami Yokota Hirai[4] and Naoko Ohkama-Ohtsu[3]

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[4] *RIKEN CSRS, Yokohama, Japan*

We have been studying the degradation pathway of glutathione (GSH) in plants (Ito and Ohkama-Ohtsu, 2023). We found that γ-glutamyl transpeptidase (GGT) 1 functions in the alleviation of oxidative stress. Based on their expression patterns, GGT1 and GGT2 were suggested to function in transporting glutathione from source to sink organs. GGT4 was found to degrade glutathione conjugates in the vacuole. Since GGTs exist outside of cells, it was hypothesized that other enzyme(s) regulate glutathione content inside plant cells. Through physiological analysis, we suggested that glutathione is degraded by γ-glutamyl cyclotransferase (GGCT) via oxoproline. Complementation analysis of yeast mutants defective in glutathione degradation using Arabidopsis cDNA revealed that γ-glutamyl peptidase (GGP) is another enzyme that degrades glutathione in cells. We propose that glutathione is constantly degraded by GGP and that GGCT assists in the degradation process under sulfur deficiency or in developing tissues where Cys is rapidly required (Ito et al., 2022). GGP was considered to have dual functions, degradation of GSH in primary sulfur metabolism and previously reported processing GSH-conjugates in the glucosinolates synthesis. To verify these dual functions, we performed ^{34}S tracer experiments using the *ggp1-1/ggp3-1* mutant. GSH was actively degraded to redistribute Cys even under normal conditions, which process was retarded in *ggp1-1/ggp3-1* compared to wild type plants.

We also identified an Arabidopsis gene, Cys-Gly dipeptidase CGP1. Analysis of the Arabidopsis *cgp1* mutant revealed that this enzyme is important for Cys supply, especially during germination in young plants (Miyaji and Ito et al., 2024).

References:

Ito T, Kitaiwa T, Nishizono K, et al. (2022) Glutathione degradation activity of γ-glutamyl peptidase 1 manifests its dual roles in primary and secondary sulfur metabolism in Arabidopsis. The Plant Joural **111**: 1626–1642

Ito T, Ohkama-Ohtsu N (2023) Degradation of glutathione and glutathione conjugates in plants. Journal of Experimental Botany **74:** 3313-3327

Miyaji S, Ito T, Kitaiwa T, et al. (2024) N^2-Acetylornithine deacetylase functions as a Cys-Gly dipeptidase in the cytosolic glutathione degradation pathway in *Arabidopsis thaliana*. The Plant Joural **118**: 1603–1618

S2-5 Contribution of sulfate transporter SULTR2;1 in plant transition from vegetative to reproductive growth through long-distance sulfate transport

Soudthedlath Khamsalath[1], Abdul wakilu Sulemana[1], Yoshifumi Goda[1], Toshiki Nakamura[1], Tsukasa Ushiwatari[1], Takehiro Ito[2,3], Jutarou Fukazawa[4], Naoko Ohkama-Ohtsu[5,6], and Akiko Maruyama-Nakashita[1]

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Sulfur (S) is an essential macronutrient for plant growth and metabolism. Plants take up sulfate from roots, transport it to shoots through xylem, and assimilate it into organic S compounds. A low-affinity sulfate transporter, SULTR2;1, facilitates root-to-shoot transport of sulfate, old-to-young leaf transport of sulfate, and sulfate transport to seeds in Arabidopsis. Its expression in vascular tissues suggested additional roles in sulfate transport. In this study, we analyzed the sulfate transport at several developmental stages using SULTR2;1 disruption lines, *sultr2;1-1* and *sultr2;1-2*, and found that sulfate distribution to the stems was affected in these mutants. As a result, these mutants decreased sulfate, cysteine, glutathione (GSH), and total S levels in the stems, flowers, and siliques. However, the GSH levels increased in the rosette leaves. To further clarify the effects of these metabolic changes in *sultr2;1*, we analysed the plant growth from the seedling to mature growth stages. *sultr2;1* unexpectedly bolted earlier than the wild-type without affecting the plant biomass. Correlation between GSH levels in rosette leaves and the bolting timing suggested that the rosette leaf GSH levels or limited sulfate transport to the early stem can trigger bolting. These results indicated the critical roles of SULTR2;1 in maintaining the S metabolite levels in the aerial part and transitioning from the vegetative to the reproductive growth phase.

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Session 3 Interactions with Other Nutrients

S3-1 Interactions of sulfur nutrition with essential micronutrients and toxic metals/metalloids

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Plant sulfur nutrition plays an important role in regulating uptake, translocation and homeostasis of essential micronutrients and detoxification of toxic metals/metalloids. Sulfate transporters facilitate the uptake and translocation of structurally similar oxyanions such as molybdate and selenate; uptake of these micronutrients is suppressed by sulfate and enhanced by sulfur deficiency. Chromate, a highly toxic metal oxyanion, is also taken up via sulfate transporters. Knockout of both *Sultr1;1* and *Sultr1;2* abolished chromate uptake by *Arabidopsis thaliana* almost completely, with Sultr1;2 playing a greater role (Xu et al., 2021). Sulfur nutrition affects the acquisition and translocation of micronutrients such as iron, copper and zinc by being involved in the biosynthesis of the metal chelators nicotianamine (NA) and mugineic acid, both of which are derived from the S-containing amino acids methionine. Mugineic acid or its derivatives secreted by roots are involved in the acquisition of iron from the rhizosphere soil, whereas NA forms stable complexes with iron, copper, zinc and cobalt for translocation to seeds via the phloem. Phytochelatins are peptides synthesized from glutathione and play a crucial role in the detoxification of cadmium, arsenic and mercury, as well as homeostasis of the essential micronutrient zinc. Formation of arsenic and phytochelatin complexes also facilitate the sequestration of arsenic in vacuoles and, consequently, reduce its translocation to seeds. Examples will be given whereby sulfur assimilation is manipulated to biofortify multiple essential micronutrients simultaneously and to reduce toxic arsenic accumulation in rice grains (Xu et al., 2024).

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S3-2 Ascorbate-Driven Chloroplastic Signaling Integrates Iron, Phosphorus, and Sulfur Deficiency to Sustain Photosynthesis

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Chlorosis and impaired photosynthesis are hallmark symptoms of sulfur, phosphorus, or iron deficiency in plants. Emerging evidence indicates that iron deficiency-induced chlorosis is modulated by the availability of other nutrients, particularly sulfur and phosphorus, yet the underlying integrative mechanisms remain elusive. Here, we investigate how plants coordinate iron and phosphorus signals to regulate chlorophyll accumulation and photosynthetic gene expression. We reveal that iron limitation suppresses photosynthesis-associated genes in a phosphorus-dependent manner (Nam et al., 2021). Through a combination of transcriptomics and genome-wide association analysis, we identify two key regulators: PHT4;4, a chloroplast-localized ascorbate transporter, and bZIP58, a nuclear transcription factor. Both genes are essential for maintaining photosynthetic gene expression under simultaneous iron and phosphorus deficiency, conferring a distinctive "stay-green" phenotype. Under joint nutrient stress, plants exhibit elevated ascorbate levels driven by the activation of the VTC4 gene, a response requiring bZIP58. We further demonstrate that chloroplastic ascorbate transport mitigates the suppression of photosynthesis genes by modulating reactive oxygen species (ROS) homeostasis. Our findings uncover a previously unrecognized retrograde signaling pathway, where ROS-mediated chloroplast-to-nucleus communication enables plants to fine-tune photosynthesis in response to complex nutrient limitations.

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S3-3 Sulfur regulates the expression of phosphorus starvation responsive genes in rice

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[Background] Sulfur (S) and phosphorus (P) are essential nutrients for plants and interact in their inter-organ transport and metabolism under their deficiencies. Nitrogen (N), another macronutrient, is known to have a strong relationship with S in metabolism and to interact with P in deficiency responses. However, the molecular mechanisms underlying the crosstalk between S and P remain largely unknown. We hypothesized that the presence of S and P is crucial for modulating their respective deficiency responses in rice. **[Methods]** [Exp. 1] Rice (*Oryza sativa* L. cv. Koshihikari) was hydroponically grown for 10 days under four different combinations of P and S treatments (−P−S, −P+S, +P−S and +P+S. −P: 0.01, +P: 0.32, −S: 0, +S: 1.22 mM). Mineral analysis and RNA-seq analysis were performed on roots and shoots. [Exp. 2] Rice seedlings were first grown for 7 days under −P−S conditions (−P was set to 0 mM). Then, seedlings were transferred to either −P+S or +P+S hydroponic solutions. In parallel, independent control seedlings were continuously grown under −P−S, −P+S, or +P+S conditions from the beginning of the cultivation. Samples from all five treatments were collected at 0, 1, and 3 days after the transfer. We conducted mineral analysis of roots and shoots, along with the expression analysis of P starvation response (PSR) genes and sulfate transporter genes in roots. **[Results]** In Exp. 1, shoot dry weight decreased under −S, regardless of P conditions, whereas −P had no significant effect under +S conditions. S and P concentrations in roots and shoots decreased in response to their respective deficiency treatments, whereas shoot N concentration decreased only under −S conditions. Genes involved in S assimilation and sulfate transport showed expression changes in response to −S, regardless of P conditions. In roots, the expression of five nitrate transporters including *OsNRT1.1B* decreased only under −S conditions. In contrast, regulators of PSR including *OsPI1* and *OsPHO2*, downstream phosphate transporters, acid phosphatases, and lipid metabolism-related genes did not show expression changes in response to −P under −S conditions. This suggests that S is crucial for regulating PSR gene expression. In Exp. 2, one day after S supplementation, S and N concentration in shoot significantly increased from pre-supplementation levels. The Expression of *OsSULTR1;1*, thought to be involved in S absorption, was higher under −P−S than under +P+S, but it decreased to +P+S levels within one day of S supplementation. The −P-induced expression of *OsPI1* was lower under −S than under +S conditions, but gradually increased following S supplementation. The expression of *OsPHO2*, a negative regulator of PSR, was significantly suppressed 3 days after S supplementation to −P−S. These results indicate that the regulation of PSR gene expression involves the mitigation of S deficiency response and possibly the alteration of rice N nutrition following S supplementation.

S3-4 Sulfur-iron interplay in durum wheat: metabolic responses and food safety.

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This study investigated the intricate interplay between sulfur (S) and iron (Fe) availability in durum wheat plants, exposing to varying Fe (20, 50, 80 µM) and S (from complete deprivation to high availability, 0, 1.2 and 2.4 mM) supply. Our findings reveal that Fe and S deficiencies differentially impact organic acid levels in wheat roots. Specifically, S starvation increased citrate levels, a rise modulated by Fe availability, highlighting a specific metabolic adjustment. Intriguingly, citrate accumulation pattern mirrored *O*-acetylserine (OAS) pattern, suggesting a coordinated response, where citrate might be involved in signaling or facilitating the S assimilation pathway under stress conditions. The impact on S metabolites like methionine and cysteine further supports this intricate interplay. The reduction of methionine with increased Fe supply under complete S starvation points to a critical link where Fe status directly influences the synthesis of this essential amino acid, which is also a precursor for phytosiderophores, crucial for Fe uptake. On the other hand, cysteine levels were lower with S deficiency, but notably, high Fe supply (50-80 µM) repressed cysteine biosynthesis to levels similar to S starvation, in contrast to the high levels observed at 20 µM Fe, suggesting a feedback mechanism or a trade-off where adequate Fe might negatively impact S metabolism.

Correlation network analysis strongly supports the central role of citrate, being its root concentrations negatively correlated with S and Fe supply, suggesting a stress-response involvement. Even more compelling are the strong positive correlations among citrate, OAS, and methionine, suggesting that these molecules might function together in a metabolic pathway or a signaling cascade that helps the plant cope with nutrient limitations.

Furthermore, our study highlights the strong impact of S and Fe supply on free asparagine levels. S deficiency consistently led to a substantial accumulation of free asparagine in both root and shoot tissues, which was further exacerbated when Fe was also deficient, highlighting a synergistic negative effect. Conversely, restoring Fe availability significantly reduced asparagine levels. This result has critical implications for food safety, as asparagine is a direct precursor to acrylamide, a neurotoxic and potentially carcinogenic compound formed during the baking process of cereal products like durum wheat.

S3-5 Cadmium stress intensity activates differential ER stress outcomes in *Arabidopsis thaliana*

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Cadmium (Cd) pollution in soils and its uptake by plant roots is a widespread problem, illustrating the requirement to enhance our knowledge of stress responses underlying its phytotoxicity. Acute Cd exposure induces an oxidative challenge in roots through the use of glutathione (GSH) for Cd-chelating phytochelatin (PC) production. To further uncover the acute Cd stress response of varying intensities, especially related to endoplasmic reticulum (ER) stress, autophagy and the ethylene response, wild-type (WT) Arabidopsis thaliana were exposed to 2 and 5 µM Cd for 24 h. Responses were explored by comparing Cd-sensitive *cad2-1* and *cad1-3* mutants with disturbed biosynthesis of GSH and PCs, respectively, to the WT. Indicative of ER stress, inositol requiring 1 (IRE1)-dependent bZIP60 splicing and target genes were induced with increasing Cd stress in roots. In leaves, however, this response was already initiated at a lower stress intensity, but also reached its limit more quickly upon increasing stress. On the other hand, IRE1-dependent decay of mRNAs (RIDD) encoding autophagy inhibitors was only induced by more severe Cd stress in both organs. Lower mRNA levels of these targets correlated with autophagy induction, pointing towards a connection between ER stress-related RIDD and autophagy upon increasing Cd stress. Furthermore, while higher stress intensity stimulated the ethylene response, it also steered the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) towards conjugation, forming malonyl-ACC. Lastly, this study demonstrates that the increased Cd sensitivity of the *cad2-1* mutant is mostly related to its lower PC production rather than its depleted GSH levels.

S3-6 Sulfur accumulation as the physiological driver of gypsum specialization

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Gypsum soils are among the most widespread extreme substrates in the world, present in 112 countries. This type of hypercalcic substrate has a set of extreme physical and chemical properties that make it stressful for plant establishment and growth. The extreme chemical properties include low plant-available nitrogen and phosphorus and high plant-available sulfur and calcium, which impose strong nutritional imbalances on plants. Despite these edaphic barriers, gypsum soils harbor rich endemic floras that have evolved independently on five continents (Moore *et al.* 2014), with highly specialized species. Plants growing only on gypsum are considered soil specialists and have a leaf elemental composition similar to the elemental availability of gypsum soils, with high accumulation of calcium, sulfur and magnesium (Palacio *et al.* 2022). In particular, they are accumulators of S, a rare phenomenon in the plant tree of life. However, the physiological and ecological role of the unique leaf elemental composition of gypsum specialists is still poorly understood, and it is unknown whether it provides an ecological advantage over other generalist species in gypsum soils (Cera *et al.* 2023). In this communication, we discuss the main insights into the impact of gypsum soil characteristics on plant life and the mechanisms underlying plant specialization in gypsum environments. We conclude with hypotheses on the potential role of sulfur accumulation in enhancing phosphorus nutrition, herbivory deterrence, and osmotic regulation as explanations for why these soil specialists are successful in gypsum outcrops.

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S3-7 Metabolic reprogramming by sulfur in the presence of selenium

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Sulfur (S) is an indispensable nutrient for metabolic and physiological processes in plants and consequently affects the production of plant primary and secondary metabolites. Thus, maintaining a sufficient sulfur supply is necessary for optimal plant performance. For instance, we recently discovered for the first time that changes in individual MGDG (monogalactosyl diacylglycerol), DGDG (digalactosyl diacylglycerol), and oxidized SQDG (sulfoquinovosyl diacylglycerol), were associated with S nutrition (Körber et al., 2023). Therefore, a greater abundance of SQDG-bearing unsaturated fatty acids and a reduction in SQDGs with saturated fatty acids are associated with higher S nutrition. Selenium (Se) is an essential nutrient for humans and animals. It regulates thyroid hormones, growth, development, differentiation, immunity, and redox homeostasis. At the same time, selenium possesses beneficial effects for plants, including alleviating the effects of biotic and abiotic stress. Sulfur and selenium share considerable physicochemical properties. Hence, selenate is taken up by the sulfate transporter and assimilated through S assimilatory pathways. This makes both elements intriguing to explore, as they influence the metabolic changes in plants, which, in turn, affect the overall quality profile of food crops. The enrichment of selenium in the presence of sulfur reprograms primary and secondary metabolism. We found that foliar application of moderate selenium along with adequate sulfur supply (via roots) is beneficial for carbohydrate accumulation and contributes entirely to the sensory characteristics of vegetable crops. Particularly, in green and red lettuce, water-soluble sugars (e.g., glucose) are enhanced synergistically. Moreover, tentative characterization of secondary metabolites was performed using (UPLC-ESI-QTOF/MS) and revealed that cyanidin 3-O-galactoside, 5-O-caffeoylquinic acid—in addition to glycosylated derivatives of quercetin (e.g., quercetin 3-O-(6''-acetyl-glucoside) and quercetin 3-O-malonylglucoside)—were detected in higher amounts in red lettuce under moderate selenium and sulfur supply (Abdalla and Mühling 2023). We can conclude that, together with sulfur application, the exact dose for the Se-sensitive vegetable crops (e.g., lettuce) should be maintained to elucidate the underlying mechanism of selenium's role as a stimulator and not a suppressor of plant biochemical and physiological events.

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Session 4 Sulfur Signaling

S4-1 Hydrogen Sulfide: An Ally in Plant Survival and Stress Resilience

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Hydrogen sulfide (H_2S), as a signaling molecule, plays an essential role in plant physiology. It regulates vital processes, such as plant responses to various stresses, particularly abiotic stresses. Thus, H_2S allows plant adaptability and viability, with beneficial effects on development. H_2S also regulates processes for adequate plant performance, including autophagy (Gotor et al., 2019).

The most well-known mechanism of action of H_2S is the posttranslational modification known as persulfidation, which was first described in plants by our research group. This PTM modifies protein cysteine residues to form persulfides, thus altering protein function. Various high-throughput proteomic analyses have revealed that persulfidation is widely distributed and identified an extensive catalog of persulfidated proteins in Arabidopsis and crops (Aroca et al., 2021). Recently, we have shown the reversibility of persulfidation by thioredoxin (TRX) systems and highlighted the mitochondrial TRX*o*1 as a depersulfidase (De Brasi-Velasco et al., 2025).

One of the significant contributions we have made is that H_2S negatively regulates autophagy through persulfidation. We have unraveled the mechanism of regulation of ATG4 and ATG18a by persulfidation of specific cysteine residues. Currently, we are interested in the role of H_2S in stress. H_2S enhances tolerance to drought, enabling plants to respond more rapidly and efficiently, through protein persulfidation. In Arabidopsis, the main role of persulfidation is alleviating the reactive oxygen species accumulation, while in rice, it is water transport activity regulation through aquaporin persulfidation.

This work was supported by ERDF A way of making Europe and MCIN/EI/10.13039/501100011033 (grant PID2022-141885NB-I00). Reyes Carrillo was supported by AEI through the predoctoral contract PREP2022-000606.

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S4-2 Novel functions of LSU proteins

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The molecular functions of short coiled-coil proteins remain largely uncharacterized; they are proposed to act as auxiliary factors supporting cellular homeostasis rather than serving as core components of metabolic pathways. They are found across a wide range of organisms, including both plants and animals. A plant-specific family of these proteins, known as LSU (UPREGULATED BY LOW SULPHUR), has attracted increasing interest. Most plant genomes encode multiple LSU-like isoforms; for example, *Arabidopsis thaliana* possesses four *LSU* genes (*LSU1–4*). LSU proteins are approximately 100 amino acids in length and contain a central coiled-coil domain. Genetic studies have shown that single, multiple, and even quadruple *lsu* knockout mutants remain viable, exhibiting only mild developmental and growth phenotypes compared to wild-type plants (Piotrowska et al., 2024a).

LSU genes are strongly induced under sulfur deficiency, and recent findings show that LSU proteins regulate sulfur assimilation by modulating enzyme activity (Piotrowska et al., 2024b). However, LSU proteins likely have broader roles beyond sulfur metabolism, acting as molecular scaffolds that influence protein trafficking, stability, complex assembly, and redox regulation through interactions with ROS-related enzymes (Niemiro et al., 2024). All known plant LSU proteins contain a conserved cysteine that likely mediates redox regulation through reversible modifications such as S-nitrosylation, S-glutathionylation, S-acylation, and disulfide bond formation, positioning them as potential redox and stress signal integrators. Overall, LSU proteins exemplify how small coiled-coil proteins can coordinate diverse cellular processes (Sirko et al., 2025).

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S4-3 Elevated Methionine Alters Sulfur Metabolism and Epigenetic Landscapes in Arabidopsis

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The key methionine biosynthetic enzyme cystathionine γ-synthase (CGS) was expressed under the control of the seed-specific phaseolin promoter in *Arabidopsis*, tobacco, and soybean. This led to a marked accumulation of amino acids and sugars in seeds, resulting in significantly elevated levels of protein and starch, which are important for nutritional value (Cohen et al. 2014). These metabolites are hypothesized to originate from leaf-derived translocation. Transcriptome profiling of *Arabidopsis* leaves revealed increased expression of genes involved in DNA methylation and chromatin remodeling. Since methionine is the precursor of *S*-adenosylmethionine (SAM)—the primary methyl donor for DNA and histone methylation—its elevated levels are expected to influence the epigenome (Girija et al. 2023). While low SAM levels have been associated with DNA hypomethylation, the consequences of increased Met/SAM remain poorly understood. To explore this, we analyzed the *mto1* mutant, which accumulates high methionine and SAM due to a mutation in CGS. Whole-genome bisulfite sequencing (WGBS) revealed widespread hypermethylation, particularly in non-CG (CHG, CHH) contexts within pericentromeric heterochromatin. Although gene body methylation (gbM) was also altered, it showed only weak association with gene expression changes. Downregulation of transposable element-associated genes (TEGs) and stress-responsive genes suggests that elevated methionine/SAM suppresses genome plasticity through TE silencing. Genes in sulfur assimilation and aspartate family pathways, which contribute to methionine biosynthesis, were downregulated, while methionine catabolic genes were upregulated. Overall, these findings establish a mechanistic link between methionine metabolism, sulfur assimilation pathway, epigenetic regulation, and nutrient allocation, highlighting the influence of sulfur metabolic status on the plant epigenome and genome stability.

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S4-4 The GTE7–IES2B-GTE2 complex epigenetically regulates sulfur homeostasis in *Arabidopsis thaliana*

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Maintaining sulfur (S) homeostasis is critical for plant growth and development, yet mechanisms regulating sulfate uptake remain poorly understood. We previously showed that the flanking sequence of the Sulfur Response Element (SURE) in the promoter of the sulfate transporter gene *SULTR1;1* display differential DNA methylation pattern under different S condition, with hyper-methylation under S-sufficient conditions and hypo-methylation under S-deficient conditions (Huang et al., 2016). We further demonstrated here that this DNA region undergoes dynamic DNA methylation in response to sulfur status, in which the hyper-methylation in S-sufficient condition could be demethylated in response to S-deficiency and the hypo-methylation could also be remethylated when resupply sulfate after S-deficiency. We identified Global Transcription Factor Group E proteins GTE7 and GTE2, along with the chromatin remodeling factor INO80 Subunit 2B (IES2B), as key regulators epigenetically regulate the DNA methylation and *SULTR1;1* expression. GTE7 but not its homolog GTE2 directly binds to *SULTR1;1* promoter and functions as a transcriptional repressor to suppress its expression. GTE7 and GTE2 exhibit functional redundancy in suppressing sulfate accumulation in shoots. The *gte7 gte2* double mutants displayed increased sulfate accumulation in shoots and impaired root growth. IES2B, a subunit of the INO80 chromatin remodeling complex, interacts with both GTE7 and GTE2, forming a regulatory complex that modulates dynamic DNA methylation at *SULTR1;1* promoter. Disruption of *IES2B* abolished S-resupply-induced DNA remethylation and elevated sulfate accumulation in shoots. Our findings reveal a GTE7–IES2B-GTE2 module that integrates transcriptional repression and dynamic DNA methylation to fine-tune sulfate homeostasis, providing insights into the complex network of transcriptional and epigenetic regulation in response to sulfur availability.

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S4-5 Sulfur-JA signaling interactions manifesting metabolic tradeoffs in Arabidopsis

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Sulfur (S) is present in essential biomolecules, such as the S-amino acids Cys and Met, the redox-active tripeptide GSH, Fe-S clusters, sulfolipids, Cys-rich defensin peptides, S-rich storage proteins and specialized S metabolites, manifesting the importance of S metabolism for plant growth and resilience. Plants control sulfate uptake and S metabolism in response to external sulfate supply and internal S demand to secure substrate S compounds for synthesizing vital components for growth and specialized S metabolites to be deployed for plant defense. Despite the importance of such requisite coordination of S metabolism for plant growth and defense, the underlying S signaling mechanisms largely remain unknown beside the established function of the SULFUR LIMITATION1 (SLIM1) transcription factor in balancing sulfate uptake and S metabolism in response to S deficiency (Maruyama-Nakashita et al., 2006). Our previous work on the Arabidopsis *jaz* decuple (*jazD*) mutant demonstrated that JASMONATE ZIM-DOMAIN (JAZ) "metabolic repressor" proteins promote plant growth and reproductive fitness but suppress jasmonate (JA)-induced immune responses, including the expression of Cys-rich defensin peptides and synthesis of defensive S-containing metabolites (Guo et al., 2018). Here we report the discovery of the *suppressor of jazD 56* (*sjd56*) mutant, which restores the growth of *jazD* but abolishes the expression of Cys-rich defensin peptides. Genetic analysis indicated that these phenotypes of *sjd56* result from the loss of a component of the Mediator complex. Comparative transcriptome analysis of *sjd56* and wild-type seedlings revealed an extensive impact of *sjd56* on metabolic S-recycling and S-sparing mechanisms, as well as plant immune responses and inhibition of shoot growth and photosynthesis under S deficiency. Our work identifies a key determinant for the transcriptional regulation of sulfate uptake and S metabolism, and further illustrates a new model for how S and JA signaling interact to control plant growth-defense balance.

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Session 5 Environmental Interactions

S5-1 Sulfur metabolites in plant microbe interactions

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Plants in their natural environments are exposed to a large range of microorganisms, some beneficial or commensal, some harmful. Plants, therefore, evolved number of mechanisms that enable them to communicate with the microbiota, to attract the beneficial ones and defend against the harmful ones. These mechanisms are mostly based on plant metabolites and many of such metabolites can fulfil both of these functions. A large number of these metabolites important for plant microbe interactions contain sulfur in their structures. The examples of such metabolites are the glucosinolates and camalexin, which are long known as part of plant immunity. However, in the last decade, the importance of these metabolites for interaction with beneficial bacteria became apparent. For example, glucosinolates have been shown to shape the composition of plant microbiome, both in roots and in the leaves. Similarly, camalexin is not only a phytoalexin defending plants against many fungal pathogens, but it is affecting root microbiome function. The rhizosphere of plant mutants in camalexin synthesis has a lower capacity for sulfatase reaction releasing sulfate from organic sources. Camalexin is also necessary for Arabidopsis to gain from interaction with some plant growth promoting bacteria. In addition, several bacterial volatiles containing sulfur, such as dimethyl disulfide, are capable to promote Arabidopsis growth. Thus, plant microbe interactions are another field in which sulfur metabolism will be playing an increasing role in the future.

S5-2 Unraveling the molecular mechanisms mediating the crosstalk between iron and sulfur homeostasis in rice.

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Plants are unique organisms capable of assimilating all the nutrients they need in elemental or inorganic forms (e.g. Fe^{2+}, SO_4^{2-}) and synthesize all the molecules required to complete their life cycle. Respiration and photosynthesis rely on iron-sulfur (Fe-S) clusters; not surprisingly, there is a wealth of physiological evidence documenting the coordination of Fe and S metabolism in plants, particularly in maize. However, and despite decades of having very detailed genomic information about model plants like Arabidopsis and crop species such as maize and rice, the precise molecular mechanisms of Fe and S sensing, and the crosstalk between Fe and S homeostatic networks, remain unknown.

Rice (Oryza sativa) is a unique species to study Fe/S interactions as despite being a grass within the Poaceae species, which uses primarily Strategy II [chelation] for Fe uptake, rice has retained both Fe uptake strategies. Retaining both Fe uptake strategies is advantageous for species that can grow in aerobic or anaerobic conditions, like paddy fields. In Arabidopsis, SULFUR LIMITATION 1 (SLIM1) has been identified as a central regulator of sulfur homeostasis, controlling ~50% of the S deficiency responsive genes. Notably, rice has two SLIM1 orthologues, and both can fully complement the phenotypes of the Arabidopsis slim mutants (hence the term ortholog), suggesting that rice TFs can form functional complexes with Arabidopsis TFs to regulate S homeostasis. At the meeting, we will present our data on the physiological characterization of the rice *slim1;1* and *slim1;2* mutants, including nutrient-specific phenotypes, gene expression and protein-protein interaction networks.

S5-3 Double trouble: combined heat stress and iron toxicity synergistically affect cellular redox homeostasis in *Arabidopsis thaliana*

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While most studies on plant stress responses focus on single stress conditions, plants in natural environments often face multiple stresses simultaneously. However, responses to combined stress conditions can often not be reliably predicted from responses to the individual stressors. As such, it is crucial to investigate how plants cope with stress combinations to enable the development of strategies to enhance plant growth and crop productivity under environmentally realistic conditions. Studying physiological plant responses to complex stress scenarios is highly challenging, as conventional methods to assess relevant physiological plant parameters are often invasive, require large amounts of plant material and/or provide only steady-state information.

Genetically encoded biosensors are interesting tools to tackle this problem, as they allow dynamic, non-invasive monitoring of key physiological parameters with high temporal resolution. As a proof of concept, we used genetically encoded redox biosensors to characterize plant responses to combined heat stress and iron (Fe) toxicity. Responses of one week-old *Arabidopsis thaliana* seedlings stably expressing cytosolic sensors for the glutathione redox potential (Grx1-roGFP2) and hydrogen peroxide (roGFP2-Orp1) were dynamically monitored in a plate reader upon exposure to combinations of high temperature (37°C) and excess Fe (50-500 µM). The results showed that both heat stress and Fe toxicity increased the glutathione redox potential and hydrogen peroxide levels in the cytosol. Interestingly, combined exposure to both stressors resulted in strongly synergistic effects on both parameters. This coincided with a significantly stronger inhibition of primary root growth. Moreover, transcript levels of genes related to glutathione metabolism and cellular redox homeostasis were differentially affected by combined heat stress and iron toxicity compared to the single stresses.

Taken together, these data suggest that plants growing on Fe-polluted soils are significantly more sensitive to high temperature in comparison to those growing under Fe-sufficient conditions and emphasize the importance of considering complex stress interactions when assessing plant stress resilience.

S5-4 Unravelling cellular communication networks and antioxidant metabolism driving stress-resilient C4 photosynthesis at cell-type resolution.

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Abiotic stresses, such as heat and excess sunlight, perturb photosynthesis in chloroplasts and induce the accumulation of reactive oxygen species (ROS). This activates stress signalling pathways that alter gene expression towards mitigating oxidative damage, which can be linked to sulphur metabolism through key antioxidants and signalling molecules, such as glutathione and 3'-phosphoadenosine 5'-phosphate (PAP) respectively. However, our understanding of chloroplast-mediated signalling is derived almost exclusively from C3 plants, with limited knowledge of how sulphur metabolism and abiotic stress signalling intersect in the biochemically- and cell type-specialised chloroplasts of C4 plants.

To address this gap, we integrated live-cell imaging, metabolomics, and single-cell transcriptomics to investigate the stress signalling networks activated in specialised leaf cell types of the C4 model species, Setaria viridis. Under heat and high light stress, we observed time- and cell type-dependent ROS accumulation patterns in mesophyll (M) and bundle sheath (BS) cells, suggesting cell-specific activation and regulation of stress signalling networks. Correspondingly, PAP, a chloroplast stress signal derived from the sulphur assimilation pathway, accumulated specifically in M cells under stress, indicating cell type-specialised induction of PAP signalling.

Transcriptomics revealed differential regulation of key sulphur metabolism genes between M and BS cells during stress correlating with cell-specialised photosynthetic machinery and suggesting that sulphur metabolism is tailored to the distinct metabolic demands of each C4 cell type. Single-cell RNA-seq data further indicated cell-specific modulation of chloroplast-to-nucleus retrograde signalling and key downstream acclimation responses, including cell-specific expression of antioxidant enzymes and ROS scavenging pathways. These transcriptional differences correlate with cell type-specialised chloroplast architecture and differential levels of ROS and PAP accumulation, illustrating how photosynthesis and abiotic stress responses are intertwined at the cellular level in C4 plants. Collectively, our results suggest that a deeper understanding of how chloroplast- and cell type-specialisation intersects with stress signalling in C4 systems may provide novel strategies for enhancing both productivity and stress resilience in bioengineered crops in the face of climate extremes.

S5-5 Investigating the role of sulfur metabolism in the beneficial interaction between *Pseudomonas argentinensis* SA190 and Arabidopsis under drought

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Drought is one of the most obvious consequences of climate change and significantly affects the biomass of crops. Finding a sustainable approach to mitigating the consequences of drought stress will help increase crop yields in agriculture. Plant growth-promoting bacteria are a sustainable option in agriculture to enhance crop yields, particularly in arid and semi-arid regions. Pseudomonas argentinensis SA190, discovered in Saudi Arabia, has been shown to improve plant performance under drought stress (Alwutayd et al., 2023). However, more information is needed about the genes and mechanisms underlying the beneficial effects of SA190. Since sulfur and sulfur-containing compounds play a key role in abiotic stress responses, several Arabidopsis sulfur mutants were used to investigate SA190's impact on sulfur metabolism under 25% PEG-induced drought stress. The findings revealed that SA190 enhanced sulfate uptake under 25% PEG stress. A buthionine sulfoximine (BSO) inhibition experiment further demonstrated that SA190 enhanced glutathione (GSH) accumulation in shoots under 25% PEG stress. Additionally, the ratio of reduced to oxidized glutathione indicated that SA190 improved the redox balance in Arabidopsis under non-stress and 25% PEG stress conditions. These findings suggest that SA190 may enhance plant drought tolerance by modulating sulfur metabolism and redox homeostasis.

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S5-6 How does sulfur nutrition affect the morphology and functioning of the nodulated root system of pea plants under water deficit?

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Grain legumes like pea can meet their nitrogen (N) requirements through symbiotic nitrogen fixation (SNF) in root nodules in interaction with soil bacteria (Rhizobia). Sulfur (S) is essential for plant growth and SNF, as it is a component of nitrogenase, leghemoglobin and ferrodoxin. Declining S emissions and deposition have led to S deficiencies in cropping systems. In addition, soil water deficit constrains pea growth and yield, SNF and uptake of nutrients like S. This prompted us to study the impact of four treatments on the morphology and functioning of the nodulated root system of pea plants in RhizoTubes (cylindrical rhizotrons): S sufficiency under well-watered (WW S+) and water deficit (WS S+) conditions, and S deficiency under well-watered (WW S-) and water deficit (WS S-) conditions. An ecophysiological structural functional analysis was conducted, focusing on the nodulated root architecture and hydromineral acquisition, using two genotypes, Kayanne and Caméor, and a knock-out mutant of the vacoular sulfate transporter PsSULTR4 in the Caméor background (*sultr4)* (Bachelet et al., 2024).

While Kayanne was previously shown to be more resilient than Caméor under water deficit, we found that this was also the case under S deficiency, which led to a greater decrease in plant and shoot N concentrations in Caméor. This reduction was attributed to constrained structural components of SNF (*i.e.,* nodule size and biomass), since the functional component (*i.e.,* N fixation efficiency) remained unchanged in Kayanne and increased in Caméor. In both genotypes, S deficiency decreased overall S concentrations in the plant and its organs. Under this condition, Kayanne reduced shoot biomass, whereas Caméor allocated more biomass to its roots, although it exhibited slower growth of the root convex hull, a proxy for soil area exploration. Water deficit, irrespective of S supply, decreased shoot and nodule growth (biomass and size), increased root biomass allocation, and increased nodule senescence. In the *sultr4* mutant, the impact of impaired vacuolar sulfate remobilization on growth and the nodulated root system was evidenced by reduced shoot area and nodule size, along with increased nodule senescence compared to Caméor, even under S sufficiency. Furthermore, under WW S-, the *sultr4* mutant showed lower shoot area, nodule biomass and shoot C and N concentrations than the wild type Caméor. The higher biomass allocation to the roots of the *sultr4* mutant suggests a compensatory response aimed at enhancing S uptake. This study highlights plant adaptation to maintaining hydromineral uptake in response to S deficiency and water deficit, and to impaired vacuolar sulfate remobilization.

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S5-7 The plastid cysteine synthase complex regulates ABA biosynthesis and stomatal closure in Arabidopsis

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Global warming intensifies drought and high-light stress periods, causing severe water loss and decreased crop yields. Stomata are tiny pores generated by guard cells, and stomatal closure is one of the most rapid physiological responses to diverse abiotic stresses, including high light and soil-water limitation. The phytohormone abscisic acid (ABA) is the dominant signal governing stomatal closure.

De novo cysteine (Cys) biosynthesis is required for ABA biosynthesis (Batool et al., 2018; Chen et al., 2019). Cys biosynthesis is initiated by the reduction of sulfate to sulfide, which occurs exclusively in chloroplasts/plastids. Then, Cys can be synthesized by the two-step enzyme reaction in the cytosol, the chloroplasts/plastids, and the mitochondria. It is catalyzed by the enzymes SERINE ACETYLTRANSFERASE (SERAT) and *O*-ACETYLSERINE(THIOL)LYASE (OAS-TL). OAS-TL and SERAT physically and dynamically interact in the cysteine synthase complex (CSC) that stimulates SERAT activity and formation of *O*-acetylserine (OAS), which limits the Cys biosynthesis rate. Higher plants possess CSCs in all subcellular compartments to enable them to fine-tune Cys biosynthesis precisely to their demands.

In this study, we uncover three novel signaling axes triggered by soil dehydration and high light stress converging on the dynamic assembly of the CSC in chloroplasts (pCSC). We demonstrate that pCSC assembly triggers ABA biosynthesis and stomatal closure in response to soil-drying signals, including sulfate and CLE25, as well as high-light-induced oxylipin OPDA. Loss of the pCSC increases sensitivity to soil-drying and impairs high-light-induced stomatal closure. Our findings uncover that the dynamic assembly of the pCSC acts as a sensor hub, integrating local and long-distance stress signals to promote stomatal closure by supplying Cys for ABA biosynthesis in guard cells. We engineered the constitutively activated pCSC, resulting in increased Cys biosynthesis and consequently more closed stomata, to generate a soil-drying resilient plant showing no growth penalty.

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S5-8 Boosting crop physiology – Alternative S-containing phytoboosters and systemic resistance inducers

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Climate change in combination with other factors, built a cultivation environment that causes reductions in crop yields due to various adverse conditions. The foliar application of natural compounds improves the obtained yield under the circumstances. Applying a range of metabolites in crops with valuing properties and functions, such as glutathione, proline, glycine betaine, citric acid, L-tryptophan, polyols, ascorbic acid, lipoic acid, and tocopherol, contributes to the crop's ability to tolerate various abiotic stresses it encounters at different developmental stages (Bouranis and Chorianopoulou, 2023). Plant resistance inducers (PRIs) are agents that lead to improved protection to pathogen attacks by inducing the plant's own defence mechanisms. PRIs are known to be effective against various pathogens (Alexandersson et al., 2016; Király et al., 2012). Such applications usually result in improved yields when applied in the field. Foliar application of plant metabolites has proven to be an effective way to support the tolerance of the crop to the various abiotic stress. Within the various tested plant metabolites so far, several S-containing compounds that seem to be of prominent importance have been incorporated in the foliar fertilization practice (Bouranis and Chorianopoulou, 2023) and alternative approaches will be discussed.

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Session 6 Applied Sulfur Technologies

S6-1 Advancing Investigation of Sulfur from the Molecular to the Global Scales

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Sulfur is an essential building block of life, required for fundamental processes, such as synthesis of the amino acids cysteine and methionine, uptake of nitrogen (N), and defense against diseases. Recent research has demonstrated that S-deficiency is a growing concern for crop systems, largely due to the loss of "free" S from atmospheric deposition, now lowered due to air quality regulation in many parts of the world. This phenomenon is creating a need for more research on S from the molecular to the global scales—not only to determine how the cells of plants and organisms use S, but also to determine where, when, and why S deficiency is occurring globally. In this talk, I will lay out a roadmap for a new, global study to: (1) quantify emerging S limitations to yields in major cropping regions globally; (2) develop time series of atmospheric S deposition and S fertilizer use by country; and (3) add S as the 4th major nutrient to the global Cropland Nutrient Balance database maintained by the International Fertilizer Association and the Food and Agriculture Organization. I will also discuss how S researchers working across scales have unique opportunities to collaborate. This project is a new effort across multiple academic, business, and nonprofit agencies, and will provide large-scale motivation for much of the research conducted by scientists attending the 13th International Plant Sulfur Workshop.

S6-2 “Cryptic” Sulfur Cycling in Hydrologically Dynamic, Vegetated Wetlands

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Traditionally, the role of sulfur in freshwater biogeochemical cycling has been underappreciated because of the relatively low concentrations of sulfate compared to in marine settings. However, recent work has shined a light on “cryptic” sulfur reactions involving intermediate-valence sulfur forms, which can drive fast redox cycling coupled to iron and carbon but often remain hidden because of small net changes in sulfate concentrations. This presentation explores “cryptic” S cycling in wetland porewaters in hydrologically dynamic, vegetated wetlands, and we demonstrate its implication on freshwater plant function, water quality, and greenhouse gas emissions. Using reactive transport modeling constrained by detailed geochemical and microbial analyses, our case studies include wetlands around the Laurentian Great Lakes that contain wild rice, a culturally significant aquatic plant for Indigenous peoples in the region, as well as a densely vegetated wetland in South Carolina (USA). In the Great Lakes wetlands, we found that rapid reduction of anthropogenically sourced sulfate drives the release of toxic sulfide for wild rice, yet groundwater upwelling can serve to buffer sulfate inputs. In the South Carolina wetland with naturally low sulfate concentrations, we concluded that coupled sulfur cycling promotes the release of iron for immobilizing toxic metals, with further enhancements from hydrological flux reversals driven by plant transpiration. In both, methane concentrations were mediated by the delivery of sulfate, which was controlled by a combination of “cryptic” sulfur cycling, variable water fluxes, and industrial discharges. Across their diverse settings and motivations, our freshwater wetland studies demonstrate how dynamic hydrobiogeochemical conditions can be both facilitated by and exert impact on plant function, with important ecosystem-wide implications when considering future climate and land-cover change.

S6-3 Using stratospheric sulfate aerosols to reduce climate change impacts: a climate and environmental science perspective

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Sulfur has a long history of human manipulation. Up to now, the two major pathways by which humans continue to influence the S cycle have been 1) industrial byproducts of combustion, that are a large source of S into the lower atmosphere, and 2) S fertilizers and other S-containing agriproducts, that are a large source of S to the biosphere. For the former, climate science has long identified the impact of the airborne sulfate aerosols, through both direct reflection of incoming sunlight and indirect increases in cloudiness, in "masking" a small portion of the greenhouse gases-driven warming. This masking, of course, has come at the expense of air quality and acid rain.

As climate change worsen and its impacts become more manifest, however, the idea to leverage the cooling potential of sulfate aerosols through their injection in the stratosphere – usually known as sulfate geoengineering, or Stratospheric Aerosol Injection (SAI) – is being discussed more and more. This idea comes from the direct observation of the large cooling that followed explosive volcanic eruptions rich in sulfate, which resulted in the formation of long-lasting sulfate aerosols in the stratosphere, reflecting sunlight and lowering global temperatures significantly.

This third, potential new pathway of human influence in the S cycle clearly comes with tradeoffs of its own, and a host of technical, environmental, political and ethical issues that need to be engaged with. Here, I will provide an overview of the state of the science of the potential climatic impacts of SAI, including methodologies to understand impacts on regional temperature and precipitation patterns and on atmospheric composition, as well as some of the ethical and geopolitical implications of a direct intervention on planetary climate, and then outline an ideal research agenda for a range of environmental impacts and uncertainties that would need to be better understood before any decision about a potential implementation (or ban) is made.

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S6-4 Sulfur application in arable crops - recent approaches

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Due to reduced atmospheric sulfur deposition in recent decades, targeted fertilization has become increasingly important to meet crop demands. Oilseed rape has the highest sulfur requirement among arable crops, typically 30–50 kg S/ha, to ensure high oil content and yields. Quality wheat needs about 10–30 kg S/ha to maintain protein levels and baking quality, while malting barley requires 10–20 kg S/ha to optimize grain quality. Potatoes benefit from 10–20 kg S/ha, improving tuber quality and starch content. Maize has a relatively low sulfur demand (5–15 kg S/ha) but may respond well to sulfur where high nitrogen rates are applied to balance the N/S ratio. In viticulture, soil-applied sulfur plays a minor role, but elemental sulfur is widely used as a fungicide against powdery mildew. Common fertilizer forms include sulfate-based products (*e.g.*, ammonium sulfate, potassium sulfate) while elemental sulfur must be oxidized by soil microbes before plant uptake. Combined nitrogen–sulfur fertilizers are widely used in practice to improve nutrient efficiency but may have interaction effects. Optimal timing and rating depends on the crop: cereals and rapeseed should receive sulfur early in spring, while potatoes and maize often get sulfur alongside nitrogen top-dressing. Current trends focus on site-specific management based on soil and leaf analysis, stabilized fertilizers to minimize leaching, and precision application to meet crop needs sustainably while protecting water and soil quality.

S6-5 The role of sulfur metabolism in shaping durian flavor during fruit ripening

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Durian (*Durio zibethinus* L.), a climacteric fruit widely cultivated in Southeast Asia, is distinguished by its strong sulfurous aroma resulting from the emission of volatile sulfur compounds (VSCs) such as methanethiol, ethanethiol, and hydrogen sulfide during ripening. These volatiles originate from sulfur metabolic pathways involving key intermediates including γ-glutamylcysteine (γ-EC), glutathione (GSH), cysteinylglycine (Cys-Gly), cysteine, and methionine. This study investigated the activation of sulfur metabolism in durian pulp during ripening, focusing on enzymatic steps and transcriptional regulation linked to VSC biosynthesis. We examined three enzymes: methionine γ-lyase (DzMGL), which catalyzes methionine degradation; Cys-Gly dipeptidase, which releases cysteine from GSH catabolites; and glutamate-cysteine ligase (DzGCL), the rate-limiting enzyme in GSH biosynthesis. Given the high levels of γ-EC and GSH in durian pulp, DzGCL is of particular interest. The HD-ZIP transcription factor (DzHD-ZIP1.8) was also investigated for its specific role in regulating DzMGL expression. This work aims to elucidate the enzymatic and transcriptional control of sulfur metabolism during durian ripening, providing insight into the molecular basis of aroma formation and supporting future strategies for fruit quality improvement.

S6-6 Enhancing seed composition to feed and fuel the world

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Seeds store energy for the growing embryo in the form of oil, protein, and carbohydrates. To produce useful products from seeds, the ratio of these macromolecules can be modulated, such as increasing protein and oil. Current commercialized seed oil traits improve oil functionality. Through combinations of genetic screens, metabolic engineering, protein engineering and gene editing we have created a new generation of quality traits that enhance nutrition or energy density of oilseeds. Specifically, we have increased the methionine content in soybean using CRISPR editing and combined it with high protein and high oil mutant lines. These seed traits can complement existing plant improvement strategies for consumer benefit.

Abstracts of Poster presentations

P1 Knockdown of β-conglycinin α′ and α subunits alters seed protein composition and improves salt tolerance in soybean

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Soybean is an important plant source of protein worldwide. Increasing demands for soybean can be met by improving the quality of its seed protein. In this study, *GmCG-1*, which encodes the β-conglycinin α′ subunit, was identified via combined genome-wide association study and transcriptome analysis. We subsequently knocked down *GmCG-1* and its paralogues *GmCG-2* and *GmCG-3* with CRISPR-Cas9 technology and generated two stable multigene knockdown mutants. As a result, theβ-conglycinin content decreased, whereas the 11S/7S ratio, total protein content and sulfur-containing amino acid content significantly increased. Surprisingly, the globulin mutant exhibited salt tolerance in both the germination and seedling stages. Little is known about the relationship between seed protein composition and the salt stress response in soybean. Metabonomics and RNA-seq analysis indicated that compared with the WT, the mutant was formed through a pathway that was more similar to that of active salicylic acid biosynthesis; however, the synthesis of cytokinin exhibited greater defects, which could lead to increased expression of plant dehydrin-related salt tolerance proteins and cell membrane ion transporters. Population evolution analysis suggested that *GmCG-1*, *GmCG-2*, and *GmCG-3* were selected during soybean domestication. The soybean accessions harboring $GmCG\text{-}1^{Hap1}$ presented relatively high 11S/7S ratios and relatively high salt tolerance. In conclusion, knockdown of the β-conglycinin α and α′ subunits can improve the nutritional quality of soybean seeds and increase the salt tolerance of soybean plants, providing a strategy for designing soybean varieties with high nutritional value and high salt tolerance.

P2 The Sulfide Symphony: Harmonizing Photorespiration, Redox Balance, and Stress Tolerance in Arabidopsis thaliana

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Hydrogen sulfide (H_2S) is increasingly recognized as a pivotal signalling molecule in planta, particularly in the context of mitigating environmental stresses associated with climatic change, including elevated atmospheric CO_2. This gasotransmitter modulates key physiological and biochemical pathways, enhancing plant resilience via mechanisms encompassing stomatal regulation, antioxidant defence systems, and osmotic adjustment. Prior evidence indicates that sulfide can ameliorate carbon/nitrogen imbalances in plants grown under suppressed photorespiration in high CO_2 environments, restore ATP levels, counteract reactive oxygen species (ROS) accumulation, and regulate stomatal closure by diminishing ROS bursts in stomata under high CO_2 (1).
Our research, utilizing wild-type and photorespiratory mutant lines of *Arabidopsis thaliana*, reveals that sulfide treatment exerts positive effects on plant growth, reduces ROS levels, and influences the content of ascorbic acid and anthocyanins, as well as regulates fatty acid metabolism. Furthermore, analyses of nitrogen assimilatory enzymes and nitrogen metabolism-deficient mutants suggest a beneficial role of sulfide in relation to photorespiration and carbon/nitrogen balance. Notably, persulfidation of specific photorespiratory proteins was observed, impacting their enzymatic activity.

This study further investigates the intricate relationship between photorespiration and stress responses, complemented by transcriptomic analysis elucidating the impact of sulfide treatment on the expression of hypoxia-associated genes under non-photorespiratory conditions in Arabidopsis.
Collectively, these findings offer novel insights into the complex regulatory mechanisms involving sulfide-mediated persulfidation in Arabidopsis photorespiration and broader metabolic processes. A comprehensive understanding of the multifaceted roles of H_2S in plant stress responses presents potential avenues for developing crops with enhanced tolerance to the escalating challenges of global climate change and increasing global population demands on agricultural productivity.

This work was supported by ERDF A way of making Europe and Junta de Andalucía, Spain (PROYEXCEL_00177).

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P3 Role of persulfidation during plant-microbe interactions

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Plants are exposed to a wide range of environmental challenges arising from both abiotic and biotic factors. To adapt to fluctuating environments, biological systems have evolved sophisticated intracellular signalling mechanisms to coordinate internal responses to external stimuli. Among these mechanisms, Post-Translational Modifications (PTMs), such as the persulfidation of cysteines (RSH → RSSH), serve as crucial regulators of protein function and cellular networks. Persulfidation has been implicated in a range of physiological processes (Wang et al., 2023), and emerging evidence suggests that persulfidation contributes to both fungal pathogenicity and host immune responses (Sueiro-Olivares et al., 2021). However, its specific role in the context of plant-microbe interactions requires further investigation. In our study, we aim to investigate the role of cysteine persulfidation in *Arabidopsis thaliana* during interactions with biotic stressors including *Pseudomonas syringae*, *Verticillium dahliae*, and *Serendipita indica*. To achieve this, we have optimised established methodologies, including both LC-MS-based (qPerS-SID) (Longen et al., 2016) and gel-based (Dimedone-Switch) techniques, to enable precise quantification of persulfidated proteins.

Preliminary data suggests that, during pathogenic challenge, the persulfidation status of various key proteins associated with photosynthesis, protein metabolism, and amino acid metabolism are differentially regulated. These findings point to a previously unrecognised layer of regulation within plant defence responses. A comprehensive analysis of this novel process may shed light on how persulfidation modulates the interplay between plant and microbial interactions.

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P4 Can Sulfur starvation responses tip the balance of plants' redox states?

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Mineral nutrients are fundamental for plant growth and development. Sulfur (S) is a macronutrient incorporated in various essential S-containing metabolites that play an important role, for example, in mitigating abiotic and biotic stress factors. Nevertheless, our understanding of how plants respond to S insufficiency and subsequent replenishment remains incomplete. We hypothesise that the insufficiency and replenishment of S affect the innate redox state of the plants since ROS is a general response mechanism of plants for combating abiotic and biotic stress. Therefore, we are using roGFP2-based biosensors to examine how sulfur availability affects the redox state in Arabidopsis roots. In parallel, we found that common transcriptional response in Arabidopsis, tomato, rice and setaria consists of only seven genes. From these seven shared genes, at least four are linked to the S starvation network, and might therefore contribute to the alterations of redox state. Thus, we explore whether the absence of these crucial sulfur starvation response (SSR) genes impacts the redox balance during sulfur deficiency and subsequent replenishment. Our initial results suggest that upon the resupply of S to S starved plants, wild-type roots get reduced from their steady state, whereas in the mutants of key SSR genes, the redox states remain insensitive to the resupply. Overall, our initial experiments indicate a possible shift in the redox equilibrium in plants under S insufficiency.

P5 *Cannabis sativa* as a model to investigate nutrient allocation and improve yields

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Cannabis (*Cannabis sativa* L., is one of the oldest domesticated plants. Selection for the production of fibre, seed and drugs has led to diverse genetic and phenotypic variation within the species (Clarke and and Merlin, 2016). Specialised metabolites within the glandular trichomes of female flowers, *i.e.*, the cannabinoids Δ^9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), are used as pharmaceutical and recreational drugs. The difference in the specific usage of hemp- and drug-type Cannabis is reflected in their growth habit and cultivation. Hemp types are grown in field conditions across a divergent range of environments and regions, often on marginal lands. By contrast, drug types are largely grown in high-input, cost-intensive protected cropping or indoor environments due to their substantial value and quality standards imposed on pharmaceutical production. Inherent differences in their nutrient requirements are important factors to optimise plant biomass and product yield (Jost et al., 2024).

We have started to develop Cannabis into a model plant to understand nutrient acquisition and allocation strategies by exploiting the diversity among Cannabis usage types (Wee Y et al., 2024). We performed RNA-seq analyses and nutrient profiling across transport, source and sink organs of Cannabis plants supplied with varying phosphate supplies. We find that acclimation to P starvation alters nutrient acquisition, especially that of nitrate and sulfate, and resource allocation towards flower development. In contrast to established crop species, Cannabis does not downregulate phosphate uptake. Our data provide a detailed understanding of phosphate starvation responses and cross-talk with N and S assimilation in Cannabis. Gained knowledge will support the development of targeted strategies for optimizing nutrient use in this multifaceted crop.

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P6 Genotypic variation in sulfur-mediated modulation of iron homeostasis in durum wheat.

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Iron (Fe) deficiency is a significant constraint on crop yields, human health, and food nutritional quality worldwide. While high sulfur (S) supply can improve Fe nutrition in some grasses, the underlying mechanisms driving this phenomenon aren't yet fully understood. This study investigated the physiological, ionomic, and molecular responses to varying Fe and S availability in four genetically different durum wheat genotypes, Svevo, Karim, LcyE $A^{-}B^{-}$, and Svems16. Fe deficiency significantly hindered plant growth and caused varying degrees of chlorosis across genotypes. Notably, high S supply mitigated chlorosis in Karim and promoted root development in most genotypes, especially Svems16. Ionomic analysis showed that Fe deficiency primarily drove nutrient shifts in roots. However, combining high S with Fe deficiency restored shoot ionomic profiles to control levels, indicating a clear mitigating effect. Total S analysis revealed genotype-specific accumulation patterns. Svevo consistently exhibited low S, potentially due to a sulfate transporter variant. Conversely, Karim showed elevated root S under combined stress, suggesting increased S channeling towards phytosiderophore (PS) biosynthesis, a finding supported by genotype-dependent PS release. identified relevant variants in methionine metabolism and PS-related genes, providing a molecular basis for the observed physiological differences. Furthermore, ATPS and OASTL activity patterns confirmed the genotype-specific role of root S metabolism in responding to Fe deficiency. Crucially, grain ionomics revealed distinct genotype-specific responses: Fe accumulation increased in LcyE $A^{-}B^{-}$ under combined high S and Fe deficiency, and in Svems16 under high S alone. In contrast, Karim, the most sensitive genotype, exhibited reduced grain Fe accumulation under Fe deficiency.

These findings underscore the diverse S-mediated strategies employed by durum wheat genotypes to maintain Fe homeostasis and identify promising targets for breeding programs aimed at enhancing nutrient use efficiency and biofortification.

P7 Sulphur limitation in marine microalgae: effects on growth, cell composition and photosynthesis

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Sulphur, an essential element in several cellular components, also plays a key role in photosynthesis, both in the electron transport and in carbon fixation reactions. Despite such central functions, our knowledge on S metabolism in microalgae is still lacunose, particularly concerning their huge phylogenetic diversity.

Here, we investigated physiological responses to prolonged low sulphate availability in three marine microalgae, the Chlorophytes *Tetraselmis suecica* and *Dunaliella salina* and the diatom *Phaeodactylum tricornutum,* selected because they belong to different phylogenetic groups. We acclimated the cultures to a sulphate concentration 500 times lower than that of the control medium (artificial seawater, mimicking the current oceans sulphate concentration of 25 mM sulphate). Characterization of their growth, photosynthesis, elemental and macromolecular composition showed that, as a general trend, all the microalgae grown in the low sulphate medium prioritized the allocation of available resources to photosynthesis. They kept their *in vivo* photosynthetic activity close to that of control cultures by modulating their pigment content per cell and the stoichiometry of their photosynthetic apparatus subunits. Conversely, growth and cell composition were modulated in a species-specific manner, with the diatom being the species most negatively affected in our experimental setup. Notably, although in present oceans sulphate is not in limiting concentration for algal growth, in ancient oceans sulphate availability was much lower and such variation was paralleled by changes in the ecological abundances of algal groups, with the red algae lineage, dominant in the present-day oceans, supplanting the green algae, more abundant in the past. The physiological responses we detected are in line with the “sulphate facilitation hypothesis”, suggesting that sulphate abundance contributed to the rise to dominance of red lineage algae.

P8 Comparative analysis of sulfur deficiency-associated metabolic responses in crop species

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Sulfur (S) is an essential macronutrient for plant growth and development, playing a crucial role in the biosynthesis of several important primary metabolites including amino acids and lipids, as well as secondary metabolites, such as the Brassica-specific glucosinolates (GSLs). S deficiency negatively affects vegetative growth, flowering and yield of seeds and fruits. Additionally, S availability significantly influences the overall plant metabolic profile and nutritional quality of plants.

To investigate the responses to S deficiency in major crop species, *Glycine max* (soybean) and *Solanum lycopersicum* (tomato), with *Arabidopsis thaliana* as a control, were grown for 21 days under two nutrient conditions: full S (1500 µM sulfate) and S-deficient (15 µM sulfate). Shoot and root samples from each plant were analyzed for primary and secondary metabolites using GC-MS and LC-MS. Results show that under S deficiency, both tomato and Arabidopsis displayed a significant accumulation of *O*-acetylserine (OAS) and increased levels of nitrogen (N)-rich amino acids, while methionine levels decreased, in agreement with previous reports (Bielecka *et al.*, 2015; Zuchi *et al.*, 2015). In contrast, soybean did not show a significant accumulation of OAS or a decrease in methionine, suggesting the presence of species-specific strategies for adapting to S deficiency. Secondary metabolite analysis revealed that Arabidopsis exhibited a decrease in S-containing GSLs under S deficiency. In contrast, soybean and tomato showed increased flavonoid levels, while terpenoid accumulation remained largely unchanged. These findings highlight the importance of analyzing regulatory mechanisms of S metabolism across multiple plant species to elucidate common and species-specific adaptation mechanisms to S deficiency.

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P9 Rice Metabolic Response to Sulfur Deficiency Conditions

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Sulfur is an essential macronutrient required for plant growth and development. It plays a critical role in the biosynthesis of sulfur-containing amino acids, cofactors, and many other secondary metabolites. Sulfur deficiency has been reported to cause yield reduction in rice. Despite this, the detailed underlying mechanisms of sulfur metabolism and its regulation in rice remains partially understood.

To characterize the metabolic response of sulfur deficiency in rice, plants were grown on control medium (1500µM sulfate) and sulfur deficiency (SD) medium (15 µM sulfate) for 21 days Samples from both control and SD plants were analyzed using GC-MS and LC-MS based metabolomic approaches. Under SD conditions, the relative abundance of the primary metabolite *O*-acetylserine, a known marker of sulfur deficiency was significantly increased compared to control plants. Similarly, nitrogen-rich amino acids as well as serine and glycine also showed increased level under SD conditions whereas methionine levels decreased. These results are consistent with previous reports in several plant species including Arabidopsis (Bielecka et al., 2015) and tomato (Zuchi et al., 2015). Secondary metabolites were also observed to respond to SD conditions, in particular, flavonoids and terpenoids, which are known antioxidants, generally showed an increased relative abundance. This metabolic shift may serve as a compensatory mechanism to maintain redox balance and support survival under sulfur deficiency.

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P10 Nutrient responses in non-model plants as a read-out of their habitat constraints

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Plants tightly control nutrient acquisition and root-to-shoot translocation with soil availability, metabolic demand and developmental programs (Collins *et al.*, 2024). To date, significant progress has been made in identifying regulatory networks and metabolic signals that synchronize these processes in model species such as Arabidopsis, tomato and rice. These species, as well as our most agronomically important crops, have evolved in environments where N frequently limits plant productivity (Prodhan *et al.*, 2019). Hence, regulatory networks are coordinating phosphate – and sulfate – acquisition according to nitrate availability. However, many species have adapted to P-impoverished landscapes, or environments co-limited by both N and P. Whilst regulatory networks are less characterized in these species, there are some interesting acclimation responses emerging.

Here, I will provide examples of these different nutrient response types, focussing on West Australian Proteaceae (Prodhan *et al.*, 2019) and *Cannabis sativa* usage types (Wee Y *et al.*, 2024). The latter also provide an example of how selective breeding for architectural and yield-related traits can impact the interaction between nutrient and hormonal signalling networks. Implications for plant selection within the protected cropping industry will be discussed.

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P11 Genetic dissection of seed sulfur-containing amino acid and protein contents in soybean

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Soybean (*Glycine max* (L.) Merr.) seeds are good protein sources. Soybean provides essential protein and amino acids for humans and animals, while sulfur-containing amino acids are very limited for soybean nutritional quality. Here, we determined the contents of protein and 17 amino acids, obtained 36 derived traits based on the protein and total amino acid contents, and derived 34 traits based on seven amino acid family groups. Furthermore, we performed a linkage analysis of the contents of 17 amino acids and 73 amino acid-derived traits based on the recombinant inbred line (RIL)-derived Kefeng No. 1 × Nannong 1138–2. Six hundred thirty-nine quantitative trait loci (QTLs) were identified, explaining 6.07–39.00% of the phenotypic variation. In addition, a GWAS of 90 indexes was conducted with a mixed linear model (MLM), and the association results showed that 1724 significant signals involving 343 SNP were mined in a natural population containing 211 soybean accessions. Combined linkage and association analysis, six loci on chromosome 3, 7, 8, 11, 12 and 14 were co-located by both the GWAS and linkage mapping. Among these loci, the significant loci on chromosome 3 are mainly related to sulfur-containing amino acid traits. Several candidate genes were identified. These findings will facilitate marker-based breeding of soybean with improved nutritional value and clarify the genetic mechanisms of sulfur-containing amino acids and protein in soybean.

P12 Nitrogen-storing amino acids are strongly increased by sulfur deficiency and less affected by nitrogen deficiency

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The interaction between sulfur and nitrogen nutrition in plants is well established, yet the underlying metabolic coordination remains poorly understood. To investigate this interaction, a greenhouse experiment was conducted using grapevine (*Vitis vinifera* cv. Regent) subjected to varying sulfate and nitrogen supply treatments. Sulfur deficiency profoundly affected shoot growth by reducing internode length. Notably, greenness loss and increased transpiration under sulfur deficiency were intensified by full nitrogen supply, indicating a strong interaction. Despite reduced photosynthesis under sulfur deficiency, assimilation partially recovered under combined nitrogen and sulfur limitation, underscoring the importance of nutrient balance over absolute availability. Amino acid profiling revealed three distinct metabolic responses. Cysteine levels remained stable across treatments, suggesting tight homeostatic control. Arginine and asparagine accumulated under sulfur deficiency, likely as nitrogen reservoirs when protein synthesis is restricted. Conversely, glutamate, glutamine, and branched-chain amino acids strongly decreased with nitrogen deficiency but increased under sulfur limitation only when nitrogen was available. These results reveal that sulfur deficiency reprograms nitrogen storage through selective amino acid accumulation. The findings highlight amino acid metabolism as a central integrator of sulfur-nitrogen nutritional status.

P13 Does LSU influence redox homeostasis in plants?

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The LSU (RESPONSE TO LOW SULFUR) protein family is composed of small coiled-coil proteins strongly upregulated under sulfur deficiency and proposed to act as stress-related hubs in *Arabidopsis thaliana*. Recent studies revealed that LSU proteins not only participate in sulfur metabolism but also contribute to redox homeostasis through multiple mechanisms. LSU1 physically interacts with iron superoxide dismutase FSD2 and stimulates its enzymatic activity, enhancing ROS detoxification in chloroplasts (Garcia-Molina et al., 2017). Moreover, LSU1 modulates the localization and aggregation of catalase isoforms CAT2 and CAT3, two key peroxisomal enzymes responsible for H_2O_2 removal, as shown by the altered condensate formation in the *lsu* knockout lines (Niemiro et al., 2024). LSU1 interacts directly with catalases, and 3D modelling indicates multiple interaction sites affecting catalase oligomerization. Although LSU loss does not markedly alter catalase activity under optimal conditions, the *lsu* mutants display increased peroxisome aggregation and changes in ROS-sensitive photosynthetic pigments under stress. These findings support the multifaceted role of LSUs in the maintenance of redox balance via protein–protein interactions and potential modulation of ROS-scavenging enzymes across subcellular compartments.

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P14 Functional Analysis of the Glutathione-Degrading Enzyme, γ-glutamyl cyclotransferase (GGCT/ChaC), Under Salt Stress

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Glutathione (γ-Glu-Cys-Gly) is an essential tripeptide found in all living organisms, including plants. Under stress conditions, glutathione plays crucial roles, such as scavenging reactive oxygen species (ROS) and participating in redox signaling (Ito and Ohkama-Ohtsu, 2023). During salt stress, hydrogen peroxide (H_2O_2) is generated and causes cellular damage, which is mitigated by glutathione. However, paradoxically, glutathione may be actively degraded under salt stress because a gene encoding a glutathione-degrading enzyme, γ-glutamyl cyclotransferase (GGCT), is highly upregulated in roots under such conditions, according to a public transcriptome database.

Interestingly, the homolog of GGCT in *Escherichia coli*, known as ChaC, has been proposed to be a cation transport regulator-like protein and is thought to regulate the activity of cation/H^+ antiporter ChaA, together with the putative cation transport regulator ChaB. In the present study, we tested whether GGCT/ChaC and ChaB are involved in the regulation of Na^+ transport under salt stress.

We first analyzed the expression of *GGCT2;1*, *GGCT2;2*, and *GGCT2;3* in *Arabidopsis thaliana* shoots and roots treated with 150 mM NaCl. Quantitative PCR revealed that *GGCT2;1* expression was strongly induced in roots by more than 150-fold. Then, we examined the expression levels of *chaA*, *chaB*, and *chaC* in *E. coli* strain BW25113 under 0, 200, and 400 mM NaCl and found that all of them were upregulated at 400 mM NaCl.

To further investigate the functions of ChaA, ChaB, and ChaC under salt stress, we constructed double mutants (*ΔnhaAΔchaA*, *ΔnhaAΔchaB*, and *ΔnhaAΔchaC*) via P1 transduction, using *ΔnhaA*, a mutant lacking the major Na^+/H^+ antiporter NhaA. Growth analysis under salt stress revealed that *ΔnhaAΔchaA* exhibited higher salt sensitivity, whereas *ΔnhaAΔchaB* and *ΔnhaAΔchaC* showed either no growth defects or even enhanced salt tolerance compared to *ΔnhaA*.

These findings suggest that ChaB and ChaC are not involved in the regulation of Na^+ transport via ChaA and that ChaC is a pure glutathione-degrading enzyme. Whether there is any adaptive significance in the upregulation of *chaB* and *chaC* under salt stress remains to be explored. Similarly, the function of plant GGCTs under salt stress may be better interpreted from the perspective of glutathione metabolism.

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P15 Sulfur-Containing Volatiles Emitted by the Root-Derived Bacterial Community Modulate Arabidopsis Growth

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Sulfur is an essential element on planet Earth. While many studies on the interaction between plants and microbes focus primarily on nitrate, phosphorus, and iron, the importance of sulfur in these interactions is still not well defined. To investigate this, we studied the role of sulfur in the communication between Arabidopsis and its microbiome. We used a 16-member bacterial community derived from the Arabidopsis root microbiome. First, we investigated indirect interaction through bacterial volatiles and their effects on Arabidopsis growth. We showed that VOCs (volatile organic compounds) emitted by this root-derived synthetic community affect Arabidopsis growth and root system architecture, while VOCs from individual strains trigger a range of different effects (Türksoy et al., 2025). In total, we detected 135 different volatiles from individual strains, with the most abundant compound emitted by the community being the sulfur-containing volatile dimethyl disulfide (DMDS). Correlation analysis predicted that several sulfur-containing compounds promote plant growth, and we demonstrated that exposure to two such VOCs—along with DMDS—indeed enhances plant growth. Additionally, we identified plant mutants that are unable to benefit from DMDS, pointing to its assimilation into S-methylcysteine as a potential mechanism of action. Moreover, we found that the growth-promoting effect of the bacterial community via volatiles depends on sulfur availability in the environment. In conclusion, our study highlights the importance of sulfur containing volatiles in plant–bacteria interactions.

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P16 Genetic dissection of low-sulfur tolerance via linkage and genome-wide association analyses in soybean seedlings

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Low-sulfur nutrient stress severely affects yield and quality in soybean production (Zhao et al. 2006; Burkitbayev et al. 2021). However, genetic studies related to soybean tolerance to low-sulfur conditions are insufficient. Here, soybean tolerance to low-sulfur conditions was evaluated according to ten traits at the seedling stage. A total of 72 quantitative trait loci (QTLs) and 103 quantitative trait nucleotides (QTNs) related to low-sulfur tolerance in soybean seedlings were detected via linkage analysis and genome-wide association analysis (GWAS) in a recombinant inbred line (RIL) population and a natural population, respectively. Among these loci, 7 codetected QTLs were identified via two methods. In addition, 3 superior parental cross combinations with the aim of improving low-sulfur tolerance have been designed across favorable alleles, which were determined on the basis of the codetected QTLs and relative values of trait phenotypes in three environments. These results provide important evidence for understanding the genetic basis of low-sulfur tolerance in soybean and may be helpful in the breeding of new soybean varieties with high tolerance to low-sulfur soil.

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P17 The Role of Arabidopsis TRP14 in Sulfur Homeostasis and Redox Regulation

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Sulfur is an essential component of all living organisms, serving as a key element in sulfur-containing amino acids, cofactors, vitamins, and disulfide bonds. Sulfur plays important roles in plant metabolism through reactive sulfur species (RSS), particularly persulfides. These function as reversible modifications of cysteine, which are involved in regulating proteins and responses to stress (Liu et al. 2025). However, the mechanism by which this reversibility is achieved remains unknown. Thioredoxins (TRXs) play a key role in redox regulation in plants, controlling enzymes through thiol–disulfide exchange reactions. Recently, the mitochondrial TRXo1 was implicated to play a role in persulfide reduction (De Brasi-Velasco et al. 2025). Thioredoxin-related protein 14 (TRP14) is a TRX-like protein with distinct redox activity involved in persulfide reduction (Liu et al. 2025). TRP14 has also been shown to regulate cystine reduction and protein cysteinylation in mammals (Martí-Andrés et al., 2024), but its role in plants remains unclear.

To identify the role of TRP14 in plants, we investigated the Arabidopsis homolog of TRP14. Sequence and structural analyses revealed a conserved thioredoxin fold, including an active site WCPDC motif. The expression of GFP-fusion constructs showed that TRP14 is present in both the cytosol and nucleus. In order to gain insight into the physiological role of TRP14, we isolated null mutants and generated overexpression lines. Phenotypic analysis revealed that the appearance of flowering buds was significantly delayed in TRP14 overexpression lines compared to wild-type plants under normal growth conditions. To further explore whether TRP14 is involved in thiol-based redox regulation, we quantified intracellular cystine and persulfide levels.

Overall, our findings suggest that Arabidopsis TRP14 modulates sulfur metabolism and redox signalling. Further analysis of the role of TRP14 under stress conditions and the identification of its downstream targets and interaction partners will advance our understanding of sulfur-mediated redox regulation.

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P18 Organelle-coordinated cysteine biosynthesis in *Arabidopsis thaliana*

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Plants assimilate inorganic sulfur to produce essential organic sulfur compounds that support growth and enhance stress tolerance. A key step in this pathway is the biosynthesis of cysteine, catalyzed by serine acetyltransferase (SERAT) and *O*-acetylserine(thiol)lyase (OASTL), which are localized in plastids, mitochondria and the cytosol. To elucidate the compartment-specific roles and physiological significance of these enzymes, we analyzed *Arabidopsis thaliana* T-DNA insertion mutants including single and multiple knockouts of the *SERAT* and *OASTL* genes. We conducted gene expression analysis, enzyme activity assays and metabolite profiling across various tissues. Our results revealed that the contributions of SERAT and OASTL to Cys biosynthesis differ substantially among organelles and vary by tissue type (Watanabe et al., 2008a, 2008b and 2018). Furthermore, immunogold labeling indicated a directional or restricted transport of sulfur-containing metabolites such as cysteine and glutathione between subcellular compartments. These findings suggest that cysteine biosynthesis in plants is controlled by a spatially coordinated, tissue-specific metabolic network that is dynamically integrated across multiple organelles.

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P19 LSU proteins boost the sulfate assimilation pathway flux in *Arabidopsis thaliana.*

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Since plants cannot move, they have developed complex ways to sense and absorb nutrients from their environment. They can adjust their growth depending on how much of each nutrient is available. Sulfur is one of the essential nutrients for plants, therefore, the pathways of its uptake and assimilation have been thoroughly researched. Considerable progress has been made in identifying the individual genes and enzymes involved, as well as understanding how these processes are regulated. However, compared to other nutrients, we still don't fully understand how plants regulate sulfur metabolism and homeostasis. LSU (RESPONSE TO LOW SULFUR) proteins are found only in plants. Their exact function has been unknown, but they were first identified during transcriptomic studies on the sulfur deficiency response in Arabidopsis. LSU proteins are thought to act as central hubs, integrating signals from environmental cues and helping plants respond to different environmental stresses.

We show, for the first time, that LSU proteins are directly involved in the primary sulfur metabolism. We found that an Arabidopsis mutant missing all four *LSU* genes (called *q-lsu-KO*; Piotrowska et al., 2024a), when grown with sufficient sulfur, shows similar changes in gene responses and metabolites levels as wild-type plants under sulfur deficiency. This led us to test whether LSU proteins interact with enzymes of the sulfate reduction pathway. Indeed, we found that LSU proteins bind ATPS1 and ATPS3 (two out of four isoforms of ATP sulfurylase), all three isoforms of adenosine 5´phosphosulfate reductase (APR), and sulfite reductase (SiR) (Piotrowska et al., 2024b). We also show in the *in vitro* assay that LSU1 increases the activity of SiR during interaction. These findings suggest that LSU proteins support the sulfate reduction process in plants.

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P20 Screening for genes responsible for glucosinolate turnover under sulfur deficient conditions in Arabidopsis

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Glucosinolates (GLs) are sulfur-containing specialized metabolites mainly found in the family Brassicaceae, including *Arabidopsis thaliana*. GLs derive toxic volatile compounds such as isothiocyanate(ITC) upon tissue damage: thus, the canonical physiological role of GLs is chemical defense against herbivores and pathogens. Recent studies have demonstrated that *A. thaliana* hydrolyzes GLs under sulfur-deficient conditions without tissue damage, and the mobilized sulfur atoms are reintegrated into primary sulfur metabolites such as Cys. It implies that endogenous GLs also function as a sulfur reservoir. In addition to the release of inorganic sulfate, GL hydrolysis potentially mobilizes the additional sulfur atom in the ITC group which is transferred to ITC–thiol conjugates, raphanusamic acid (RA), and finally assimilated into Cys (Sugiyama et al., 2021). However, detailed pathways and physiological significance of the catabolic reactions remain elusive.

For better understanding of the molecular mechanisms underlying sulfur recycling from GLs, we aimed to explore the genes/enzymes responsible for GL degradation under sulfur deficiency, mainly focusing on the Cys regeneration reactions from RA. In this presentation, we report our attempts to screen total cDNA of *A. thaliana* for the relevant genes and validate their functions.

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P 21 'Cys'temic signaling in excess light-induced responses in *Arabidopsis thaliana*

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In plants, rapid systemic stress responses involve reactive oxygen species (ROS) waves that propagate throughout the organism in response to local stimuli, such as excess light (EL), heat and injury. While live imaging using fluorescent probes and redox-sensitive reporters like roGFP1 have demonstrated these systemic ROS waves, their impact on protein function at the molecular level remains poorly understood.

Protein cysteinyl thiols undergo dynamic oxidation-reduction reactions triggered by hydrogen peroxide, the most stable ROS, that significantly alter protein function and act as key regulators in cel lular signaling. Recent studies highlighted the importance of cysteine *S*- sulfenylation in systemic signaling, such as the modification of the transcription factor CCA1 HIKING EXPEDITION during systemic acquired resistance. However, a comprehensive analysis of cysteine oxidation dynamics in plant systemic signaling is still lacking.

Precise quantification of cysteine oxidation is critical for elucidating the mechanisms of protein redox regulation. Mass spectrometry-based methods, such as OxICAT, iodoTMT, and CysQuant, have been developed to quantify cysteine oxidation states with high accuracy and sensitivity. These advanced techniques allow researchers to map the redox landscape of proteins and investigate their functional roles in stress responses.

Here, we employ a 'turn-on' fluorescent probe for protein *S*-sulfenylation and an upgraded CysQuant method to study rapid systemic stress signaling, with a focus on cysteine oxidation, termed 'Cys'temic signalling, during EL-induced systemic responses in *Arabidopsis thaliana* leaves. We demonstrate that EL stress triggers a systemic wave of protein *S*-sulfenylation. Surprisingly, CysQuant proteomic analysis reveals more pronounced oxidation changes in unstressed systemic leaves compared to locally stressed leaves. This finding highlights the critical role of cysteine oxidation in systemic stress responses and underscores the need for further elucidation of redox signaling mechanisms in plants.

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P22 Turning Waste into Resilience: Microbial Production of Selenium Sulfide and the Impact of Selenium Species on Plant Redox Balance

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Selenium sulfide, widely known as the active compound in traditional anti-dandruff shampoos, is conventionally synthesized via the reaction of selenium dioxide (SeO_2) with hydrogen sulfide (H_2S) under acidic conditions (Zhu Shihui, Zhu Shiming, 2016). Interestingly, this process can be carried out using H_2S generated by sulfate-reducing bacteria (SRB). These robust anaerobic microorganisms not only thrive on conventional culture media but also flourish in unconventional and sustainable substrates—such as fermented waste mixtures composed of cabbage juice and compost, or spoiled milk and mineral water. In our research, SRB have been employed to utilize naturally occurring DL- lactate and sulfate (SO_4^{2-}) and generate H_2S, achieving concentrations of up to 4.1 mM in the headspace of standard cultures. This biogenic gas can be harvested and directed into a separate reaction chamber containing SeO_2, leading to the direct precipitation of selenium sulfide (Safinazlou *et al.*, 2025). This approach eliminates the need for complex purification steps, offering a sustainable route to produce high-value chemical compounds from low-value waste streams. The microbial "gas harvesting" strategy not only reduces environmental burden but also presents an innovative platform for green chemistry and industrial biotechnology.

Following its application in pharmaceutical or personal care products, selenium sulfide may eventually make its way into the environment, particularly soils, where it can be transformed into soluble selenium and sulfur species. While the roles of sulfur in plant nutrition, metabolism, and stress tolerance are well understood, selenium remains a lesser-explored element with intriguing potential, especially regarding climate change and heat and drought stresses associated with it. In our ongoing research, we investigate the uptake of selenate and selenite in *Arabidopsis thaliana* and their influence on redox homeostasis, using roGFP-based biosensors and phenotyping under both normal and high temperature conditions.

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P23 Effect of hydrogen sulphide on growth and metabolite production in herbaceous legume

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Plants employ various defence strategies like triggering immune pathways and accumulation of defense metabolites to circumvent different stress encountered in nature. In addition to the self- defense based mechanisms adequate supplementation of nutrients induces multifaceted reactions triggering signalling pathways to activate defence responses including the anti-oxidative machinery and the synthesis of specialized metabolites to counteract the stress conditions. Sulphur and its associated metabolite compounds (cysteine, glutathione, glucosinolates, phytoalexins, gaseous H_2S) are reported to mitigate stress conditions in plants by various strategies. In the recent years, H_2S in plants has been recognised as one of the crucial gaseous signalling molecules with multiple regulatory roles in plant growth, development and in mitigating stress conditions. *Trigonella foenum graecum* (Fenugreek) is an herbaceous legume that synthesize a repertoire of specialized metabolites with medicinal and nutraceutical properties. The current study aims in understanding the potential role of hydrogen sulphide on fenugreek plant physiology and metabolite production. Sodium hydrogen sulphide (NaHS) treatment didn't show any significant changes in fenugreek seedling parameters in In- vitro assays. While in pot studies after 45 days an increase in fenugreek root and shoot length were observed above 100 µM NaHS. L-cysteine desulfurase (LCD) is the major enzyme involved in H_2S production in plants. As a result, a constant increase in the LCD enzyme activity was observed up to 100 µM NaHS and the activity remained constant in the later treatments. Similarly, endogenous H_2S levels were also increased till 100 µM NaHS treatment. We could also establish an increase in production of secondary metabolites in leaves including Phenolic compounds, Saponins and alkaloids. Further the impact of hydrogen sulphide on S-metabolites and gene expression of metabolite pathways will be discussed.

P24 Deletion of GGCT2;1 suppresses the dwarf phenotype of *zir1* and inhibits camalexin production

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The tripeptide glutathione (γ-Glu-Cys-Gly; GSH) is the most abundant low molecular weight thiol in plants. It plays a key role in the detoxification of potentially toxic metabolites, and in the biosynthesis of sulfur-containing secondary metabolites, such as camalexin and glucosinolates. It is also involved in cellular redox homeostasis due to its ability to change between the reduced form, GSH, and the oxidised form, glutathione disulfide (GSSG). The redox potential (E_{GSH}) of the GSH/GSSG pair is closely linked to the protein thiol status via glutaredoxins, thus potentially modulating redox-sensitive cellular functions (Meyer *et al.,* 2021).

In Arabidopsis, root growth is well known to be dependent on glutathione. Mutants with severe GSH-deficiency exhibit stunted root phenotypes, suggesting a role for GSH directly or indirectly regulating the cell cycle in the root meristem (Vernoux *et al.,* 2000). Among other enzymes, γ-glutamylcyclotransferase2;1 (GGCT2;1) has been reported to be involved in cytosolic GSH degradation, particularly in root tips exposed to sulfur deficiency (Joshi *et al.,* 2019). Although a link between glutathione and root growth is apparent, the way in which this occurs at the molecular level remains unclear. Specifically, it is not known whether primary root growth depends on E_{GSH} or on the absolute amount of GSH. To address this question, we used redox-sensitive GFP2 (roGFP2) as a biosensor for E_{GSH}, along with mutants impaired in either GSH biosynthesis or the degradation of GSH, and investigated the consequence of combining such mutants.

Notably, deletion of GGCT2;1 suppresses the dwarf phenotype of the GSH-deficient *zir1* mutant and restores root growth to wild-type levels, highlighting how GSH turnover directly impacts developmental outcomes. Simultaneously, we observed constitutive camalexin production in *zir1*, but not in *zir1 ggct2;1* double mutants. These results suggest that GGCT2;1 may have dual functions in GSH degradation and the biosynthesis of sulfur-containing secondary metabolites.

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P25 The contribution of sulfur in crop biofortification schemes – The effect of S-containing amino acids coupled with ethoxylated surface active agents on the morphological traits of durum wheat spike

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Durum wheat is the second most cultivated species of wheat after common wheat, and the species is the hardest of all wheat types, referring to the resistance of the grain and in particular of the starchy endosperm to milling. The morphology of the inflorescence, termed the spike, is crucial in determining grain yield. The components of the spike (e.g. spikelets), influence each other. The number and arrangement of the spike components affect spike length, spike weight, spikelet number per spike, grain number per spike, and grain weight per spike, all contribute to final grain yield per spike (Guo et al. 2018). Does the foliar application of S-containing amino acids, coupled with ethoxylated surface active agents, affect the spike morphology traits?

Sowing (Don Mattero variety) took place on Dec 22, 2021, treatments on May 06, 2022 (stage Z80) through foliar application, and harvesting on June 26, 2022. Cysteine (Cys 5 mM) and methionine (Met 5 mM) were applied alone or with the following surfactants: SW7, an organosilicon-based ethoxylate (SiE) surfactant, and Saldo, an isodecyl alcohol ethoxylate (IAE) one. The results showed that in almost any application case the spike length was reduced significantly or tended to reduce, thus resulting in a denser spike. In contrast, the weight per spike remained unaffected. Per spike, Cys and Met reduced all spike morphological parameters significantly, whilst the combinations of Cys or Met coupled with SW7 or Saldo restore them. Especially, the combination of Met/Saldo increased weight per grain by 9% and the spike weight per cm by 16%. SW7 applied alone increased the spike weight per cm by 25%.

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P26 NAPstars, a novel NADPH:NADP$^+$ fluorescent-protein based biosensor family, reveal in vivo mechanisms of antioxidant defence and stress metabolism

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Nicotinamide dinucleotide phosphate (NADP) serves as a redox cofactor supporting metabolism and signalling in all living cells. As a backbone of redox signalling and antioxidant defence NADP plays a dual role in providing electrons for the generation of reactive oxygen species (ROS) via NADPH-oxidases as well as for their detoxification through the Cys-based antioxidant redox systems.
While the biochemical significance of NADP redox status in plant cells is well established, it's *in vivo* changes at the subcellular level remain remarkably poorly described, mainly due to challenges in measuring it specifically and dynamically *in situ*.
I will present the recent progress that we have made in developing a fluorescent protein-based sensor family, named NAPstar, for NADPH:NADP$^+$. Plant lines expressing the NAPstars allow for dynamic monitoring of NADP redox dynamics. I will highlight the use of the NAPstars to shed light on several fundamental questions, such as Cys-based antioxidant defence and metabolic regulation under biotic stress.

P27 Mass spectrometry-guided stable isotope tracer experiments for the analysis of glucosinolate catabolism under sulfur decifiency

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Stable isotope tracer experiments have been a powerful strategy to reveal the metabolic flow in living organisms, especially in combination with mass spectrometry-based metabolomics. Recently, we clarified that endogenous storage of glucosinolates, a class of sulfur-rich plant metabolites, is exploited as a sulfur source in *Arabidopsis thaliana*. HPLC–MS/MS analysis coupled with ^{34}S or deuterium-labeled glucosinolates depicted the sulfur flow connecting glucosinolates to primary metabolites such as Cys at the atomic level, in which sulfur is mobilized not only from sulfate but also from the thioglucoside group. This finding demonstrated the bidirectional interaction between primary and specialized metabolism (Sugiyama et al., 2021). However, it remains challenging to efficiently introduce stable isotopes into specialized metabolites as well as to comprehensively quantify incorporation of stable isotopes into sulfur-containing metabolites.

Here, we report recent advances and challenges in the stable isotope tracer experiments focusing on the glucosinolate turnover in the family Brassicaceae.

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P28 Root expansion induced by S limitation and mild heatwaves mitigated yield loss under severe heatwaves in temperate grasslands

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Grasslands are the main agricultural land use in Europe and provide several ecosystem services, such as fodder supply. However, the sustainability of grasslands is compromised by global change. Heatwaves and Sulfur (S) limitation are two common threats in Europe, which have never been analysed together. S nutrition is essential for plant response to abiotic factors and, in particular, for mitigating the effects of heatwaves by S-containing compounds. We hypothesised that S-limitation may compromise plant responses to heatwaves and compromise yield in temperate grasslands.

We conducted a greenhouse experiment to simulate the effects of heatwaves on a sown and mown grassland without water limitation, and with or without S-limitation. We designed four thermo-protocols: i) control ii) mild heatwaves alone, iii) a single severe heatwave alone, and iv) a recurrent scenario with mild and severe heatwaves. We analysed yield and heatwave sensitive leaf parameters such as leaf temperature and maximum quantum efficiency of Photosystem II.

Negative effects of heatwaves were only observed in pots with standard S nutrition, but not in S-limited pots. S-limited pots were more productive than pots with standard nutrition. S-limited pots were more productive than standard S-supplied pots, especially in the recurrent scenario. S-limited pots showed a higher root mass than standard S-supplied pots, especially in the mild and recurrent heatwave scenarios. Consequently, S-limited plants showed a lower T°C under recurrent events, which mitigated the effects of heatwaves.

S-limitation seems to stimulate root expansion, but only in mild heatwave situations. This nutrient limiting condition led to indirect priming effect that helped the plants cope with severe heatwaves. Indeed, the plants developed a wider root system that contributed to leaf cooling and to mitigate transpiration which eventually helped cope with heatwaves effects.

These results open debate on the role of S in plant responses to abiotic factors. In our case, S limitation induced unexpected heatwaves tolerance through its direct consequence on root expansion which pointed out its beneficial effects that contrasts with the hypothesised role of S-containing compounds in mitigating the negative effects of abiotic factors.

P29 The inhibition of vacuolar sulfate efflux affects leaf metabolism and triggers a stay-green phenotype through inhibition of senescence in pea

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Sulphur (S) is an essential macronutrient for seed yield ad quality in legumes. In the pea crop (*Pisum sativum*), a single gene encoding a vacuolar sulphate transporter, PsSULTR4, was identified. Its physiological role was investigated using two TILLING mutants (Caméor cultivar) for this gene which were phenotyped under S sufficiency and S-deficient conditions during the reproductive period. Under S deficiency only, the mutants exhibited a significant reduction in seed number, associated with altered S partitioning, with S being retained in the lower (vegetative) leaves (Bachelet et al., 2024). To study the physiological consequences of this sulphate remobilization defect, transcriptomic and metabolomic analyses were conducted from vegetative leaves sampled 25 days after appearance of the first flower (*i.e.,* seed filling stage in the first pods). These omics analyses revealed repression of chlorophyll degradation pathways and down-regulation of senescence-associated transcription factors, together with significant shifts in aspartate metabolism, in the *sultr4* mutant leaves. These molecular changes were accompanied by higher chlorophyll content and sustained photosynthetic activity in the lower leaves throughout the reproductive phase. Depodding experiments confirmed that impaired vacuolar sulphate remobilization, not reduced seed number, underlies this phenotype. At the end of this experiment, a stay-green phenotype was observed in the *sultr4* mutants under S deficiency, confirming the delayed senescence *in planta* under greenhouse conditions. The precise link between vacuolar sulphate efflux and senescence regulation remains to be clarified but opens new perspectives on the coordination between the vacuolar sulphate store and leaf ageing in legumes.

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P30 Disruption of sulfur deficiency-responsive microRNA, miR395, suppressed sulfur assimilation and plant growth under sulfur deficiency.

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Sulfur deficiency (−S) induces specific microRNA(miR), miR395, expressed from six loci located on the first chromosome of *Arabidopsis thaliana*. −S-inducible expression of miR395 is regulated by SLIM1 transcription factor that governs −S responses in Arabidopsis (Kawashima et al., 2009). miR395 targets several transcripts of S assimilatory proteins, a sulfate transporter SULTR2;1, and ATP sulfurylases (ATPS), ATPS1 and ATPS4, which catalyze the initial steps of S assimilation. As S assimilation is typically enhanced under −S, it remains unclear why miR395 targets S assimilatory genes, and how its disruption affects plant growth and S metabolism under −S.

We generated multiple miR395 mutants with genome editing. We first confirmed that miR395 disruption increased the transcript levels of target genes. In addition, these mutants reduced their growth, the levels of glutathione, glucosinolates, and S in protein, which was consistent with the previous report showing increased GSH levels in miR395 overexpression lines (Kawashima et al., 2011). These results suggested that miR395 stimulates S assimilation and supports plant growth under −S. We are now conducting RNA-seq analysis to assess the effects of miR395 disruptions on S metabolism and other pathways to clarify the reason for decreased S assimilation and plant growth, specifically under −S.

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P31 Contribution of sulfate transporter SULTR2;1 in plant transition from vegetative to reproductive growth through long-distance sulfate transport

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Sulfur (S) is an essential macronutrient for plant growth and metabolism. Plants take up sulfate from roots, transport it to shoots through xylem, and assimilate it into organic S compounds. A low-affinity sulfate transporter, SULTR2;1, facilitates root-to-shoot transport of sulfate, old-to-young leaf transport of sulfate, and sulfate transport to seeds in Arabidopsis. Its expression in vascular tissues suggested additional roles in sulfate transport. In this study, we analyzed the sulfate transport at several developmental stages using SULTR2;1 disruption lines, *sultr2;1-1* and *sultr2;1-2*, and found that sulfate distribution to the stems was affected in these mutants. As a result, these mutants decreased sulfate, cysteine, glutathione (GSH), and total S levels in the stems, flowers, and siliques. However, the GSH levels increased in the rosette leaves. To further clarify the effects of these metabolic changes in *sultr2;1*, we analysed the plant growth from the seedling to mature growth stages. *sultr2;1* unexpectedly bolted earlier than the wild-type without affecting the plant biomass. Correlation between GSH levels in rosette leaves and the bolting timing suggested that the rosette leaf GSH levels or limited sulfate transport to the early stem can trigger bolting. These results indicated the critical roles of SULTR2;1 in maintaining the S metabolite levels in the aerial part and transitioning from the vegetative to the reproductive growth phase.

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P32 Mechanistic control of sulfate-induced ABA biosynthesis in guard cells during drought stress

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As sessile organisms, plants face biotic and abiotic stressors that impair growth and yield on site. The most severe stress impairing seed yield is drought stress. Drought stress triggers adaptive responses that integrate enzymatic cascades and phytohormonal signaling to maintain homeostasis. In plants, stomatal closure is a key response that limits transpirational water loss and is modulated by abscisic acid (ABA) and sulfate availability (Malcheska et al., 2017) in response to changes in soil water availability. During an early water deficit, sulfate mobilized from the roots is assimilated into cysteine within guard cells (Batool et al., 2018), thereby enhancing ABA biosynthesis and stomatal closure. However, the molecular mechanism linking sulfate-induced cysteine production to ABA synthesis and stomatal regulation remains to be elucidated.

In this study, the reference plant *Arabidopsis thaliana* was exposed to exogenous sulfate to mimic root-to-shoot signaling in response to soil drying. Stomatal aperture was quantified in sulfate-treated epidermal peels to determine the right timepoint for analysis of key genes in the ABA biosynthesis pathway. We focused this analysis on the enzyme NINE-CIS EPOXYCAROTENOID-DIOXYGENASE 3 (NCED3), which catalyzes the rate-limiting step of abscisic aldehyde production, and ABSCISIC ALDEHYDE OXIDASE 3 (AAO3), which converts the abscisic aldehyde to the hormone ABA. Since the transcription factor NGATHA has been previously shown to control drought-induced ABA biosynthesis (Sato et al., 2018), we included an NGATHA loss–of–function mutant (*miRNA:NGA1-4)* in our study.

We found that sulfate treatment induced significant upregulation of NCED3 transcript levels and increased AAO3 activity in leaves, resulting in accelerated stomatal closure. Remarkably, the *miRNA:NGA1-4* mutant failed to close stomata upon sulfate application. Our findings suggest that a sulfate-driven signaling cascade induces the transcription of ABA biosynthesis key genes, potentially in an NGATHA-dependent manner, and that the induction of these key genes is essential for sulfate-induced stomatal closure.

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P33 A Lethal Arabidopsis Mutant Implies Regulation of Defense Signaling via Glutathione Degradation

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Glutathione (γ-Glu-Cys-Gly) plays multiple roles in plants, including antioxidation, detoxification, signal transduction, and sulfur storage. Despite its importance, glutathione is continuously degraded even under non-stress conditions, highlighting the significance of the glutathione degradation process (Ito and Ohkama-Ohtsu, 2023). Among glutathione-degrading enzymes, γ-glutamyl cyclotransferase (GGCT), a cytosolic enzyme, and γ-glutamyl transpeptidase (GGT), an exoenzyme, are conserved across a wide range of plant species. However, the functions of GGCT and GGT remain largely unknown, likely due to the multifunctionality of glutathione and the complexity of the glutathione degradation pathway.

In this study, we created an Arabidopsis double mutant, *ggct2;3/ggt1*, and found its lethal phenotype. *ggct2;3/ggt1* grew normally for 1-3 weeks, after which it developed a lesion-mimic phenotype and eventually died before seed set. In transcriptomic analysis, GO terms related to defense response were enriched during the propagation of cell death, including signaling pathways for salicylic acid (SA), jasmonic acid, and ethylene. At the same stage, the glutathione levels in *ggct2;3/ggt1* were more than twice compared to those in the wild type, although the reduced (GSH)/oxidized (GSSG) glutathione ratio remained comparable between them.

We also made notable observations in the *ggt1* single mutant. *ggt1* highly expressed the SA marker gene *PR1* under non-stress conditions, and *ggt1* leaves exhibited hypersensitivity to L-serine treatment. These findings also implied that the disruption of *ggt1* perturbs defense signaling in Arabidopsis.

Together, this study highlights the hidden link between glutathione degradation and defense signaling in Arabidopsis. Because excessive defense response is a typical cause of cell death and lesion-mimic phenotypes, the lethality of *ggct2;3/ggt1* is likely attributable to dysregulated defense signaling. Although the mechanism linking glutathione degradation to defense signaling remains to be elucidated, one factor may be the apoplastic glutathione concentration, which may activate defense response via glutamate receptor-like (GLR) proteins (Qi et al., 2006; Li et al., 2025). Further analysis of *ggt1/ggct2;3* and *ggt1* would uncover an overlooked mechanism of defense regulation via glutathione degradation.

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P34 Genetics of the plastic response of pea seeds to sulphur deficiency

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The renewed interest in plant proteins has stimulated research aimed at developing markers to assist in the selection of legume varieties with improved seed nutritional value. Among the traits targeted for improvement and stabilization is seed protein composition, a key determinant of the seed amino acid balance that is strongly influenced by sulphur (S) nutrition. The objectives of this study were: (i) to explore the variability in response to sulfur deficiency across a collection of pea ecotypes by evaluating yield components and seed protein composition, and (ii) to identify, through genome-wide association studies (GWAS), genes potentially involved in this variability of response, using genotyping data from PIA-PEAMUST (Aubert et al., 2023) and ANR-GRaSP. Two experiments were conducted over two consecutive years, involving 304 and 198 pea ecotypes, respectively, grown under two sulphur conditions: sufficient and deficient. Plasticity indices were calculated to reflect the magnitude of the response to sulfur deficiency for each trait. GWAS results revealed genomic hotspots and candidate genes associated with the response of seed protein composition and one-seed weight to variations in sulfur availability. These findings offer promising avenues for breeding strategies aimed at stabilizing seed weight and seed protein composition under fluctuating sulfur conditions.

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P35 The GRAS1-bHLH84 axis regulates transcription of *SULFITE REDUCTASE* to enhance glutathione accumulation and adaptation to low phosphorus stress in potato

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Low phosphorus (LP) stress is an economically relevant abiotic stress limiting plant growth in diverse agricultural systems. Thus, understanding the molecular mechanisms underlying the LP stress response is a central focus of modern plant stress physiology. Previous studies have revealed that LP-induced transcriptional activation of glutamate-cysteine ligase (*StGSH1*), which catalyzes the rate-limiting step in glutathione (GSH) biosynthesis, is critical to cope with LP-induced oxidative damage and growth inhibition (unpublished data). In the light, GSH production is limited by the final product of the sulfate assimilation pathway, cysteine. Sulfite reductase (SiR) catalyzes the reduction of sulfite to sulfide, which can limit sulfate assimilation rates under stress (Feldman-Salit et al., 2019). In this study, we addressed the importance of SiR regulation to cope with severe LP stress in the crop potato.

We found that SiR is encoded in potato by a single copy gene (*StSiR*), whose gene product localizes to the chloroplast. The *StSiR* transcript level was rapidly upregulated under LP stress in potato leaves and reached a plateau after 48 hours of phosphorus limitation. Based on this finding, we genetically engineered plants with altered SiR levels. Silencing of *StSiR* decreased sulfate assimilation and impaired growth under LP stress. Overexpression of *StSiR* (OE2, OE3) in potato resulted in significantly enhanced tolerance to LP stress, which was accompanied by the accumulation of cysteine and glutathione, resulting in an enhanced reactive oxygen species (ROS) scavenging capacity.

Although StSiR has been implicated in regulating potato resistance to LP stress, the mechanism underlying its transcriptional induction under LP remains unknown. Analysis of the *StSiR* promoter region and subsequent yeast one-hybrid (Y1H) screening of a potato leaf cDNA library using this promoter as bait identified six candidate transcription factors. Among these, only StbHLH84 was significantly induced in leaves under LP conditions and was demonstrated to activate *StSiR* transcription directly. Remarkably, 15 putative GRAS-binding cis-elements are present in the *StbHLH84* promoter. Indeed, we found that LP stress induces *StGRAS1*, which transcriptionally activates *StbHLH84*. This enhanced StbHLH84 expression, in turn, promotes the transcriptional activation of *StSiR*. This cascade stimulates cysteine biosynthesis, leading to GSH accumulation, elevated antioxidant capacity, and ultimately improved adaptation to LP stress.

In summary, our study uncovers a substantial crosstalk between the phosphorus and sulfur assimilatory pathways, which is critical for the adaptation of potato to LP stress. Our findings strongly suggest a molecular framework by which the GRAS1-bHLH84 axis transcriptionally activates *StSiR*, and *StSiR* together with *StGSH1* coordinates glutathione biosynthesis, thereby promoting potato adaptation to LP stress.

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P36 Compartment-specific cysteine production directs sulfur into specific stress responses in *Arabidopsis*

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Cysteine is the end product of the assimilatory sulfate reduction pathway and the limiting precursor for the synthesis of the stress-associated Redox buffer glutathione (GSH). Cysteine synthesis is tightly controlled by the dynamic association of the cysteine synthase complex (CSC), which is formed by the interaction of serine acetyltransferase (SERAT) and *O*-acetylserine (thiol) lyase (OAS-TL) in the cytosol, the mitochondria, and the chloroplast. Remarkably, the CSC acts as a molecular switch controlling net cysteine production within a subcellular compartment (Sun et al., 2021). Under sulfur limitation or heavy metal stress, plants must allocate cysteine either to protein synthesis or defense-related molecules like GSH (Speiser et al., 2018). It is well established that the dynamic assembly of the CSC fine-tunes cysteine production rate by controlling the synthesis of OAS. However, how the net cysteine synthesis flux is coordinated between the subcellular compartments of the plant cell under fluctuating environmental conditions remains unclear.

In this study, we introduced point mutations into diverse OAS-TL isoforms to abolish their catalytic activities while retaining their ability to interact with SERAT, thereby stabilizing the CSCs in the different subcellular compartments of the reference plants *Arabidopsis thaliana*. Genetic engineering of a stable CSC in the chloroplast markedly elevated OAS, cysteine, and GSH steady state levels and induced stomatal closure. Surprisingly, stable CSC formation in the cytosol also caused massive accumulation of OAS and thiols but did not affect closure of the stomata, while stabilizing the mitochondrial CSC had only a minor impact on net cysteine synthesis. These results strongly suggest that compartment-specific cysteine synthesis rates dictate how sulfur is channeled into cysteine to cope with specific stress responses.

To test this hypothesis, we challenged the transgenic lines with genetically engineered CSCs in the diverse subcellular compartments with Cd-stress. To cope with elevated Cd, plants must channel substantial amounts of cysteine into GSH, which serves as the precursor for phytochelatin biosynthesis in the cytosol. Since phytochelatins are sequestered with Cd into the vacuole for detoxification of Cd, Cd stress causes a substantial sink for GSH in this subcellular compartment. As hypothesized, we found that the different transgenic lines displayed distinct tolerances to Cd depending on the subcellular origin of the elevated cysteine synthesis.

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P37 A GLUTAMATE CYSTEINE LIGASE gene *StGSH1* regulated by StERF10 enhanced glutathione accumulation and the adaptation to low phosphorus stress in potato

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Potato (*Solanum tuberosum* L.) is a globally pivotal food crop whose growth and development is increasingly constrained by soil-available phosphorus deficiency. Although glutathione (GSH) modulates abiotic stress responses, its contribution to potato adaptation to low-phosphorus (LP) stress and the underlying regulatory circuitry remain undefined. Here, we demonstrate that LP stress elicits a rapid and sustained accumulation of GSH, driven by transcriptional up-regulation of GLUTAMATE–CYSTEINE LIGASE (*StGSH1*), the rate-limiting enzyme in GSH biosynthesis. Pharmacological inhibition with buthionine sulfoximine (BSO) and complementation via exogenous GSH corroborate a causal role of GSH in LP tolerance. Sub-cellular localisation of StGSH1-GFP fusions in potato protoplasts confirmed exclusive plastidial targeting. Overexpression of *StGSH1* maintained reactive oxygen species (ROS) homeostasis, attenuated LP-induced oxidative damage and rescued biomass reduction. Mechanistically, StGSH1 orchestrated membrane lipid remodelling by simultaneously up-regulating phospholipid catabolic genes (*StNPC/PLD*) and sulfolipid anabolic genes (*StSQD1/2*), thereby redirecting phosphatidylethanolamine (PE) towards sulfoquinovosyldiacylglycerol (SQDG) synthesis and enhancing phosphorus use efficiency. Conversely, *StGSH1* suppression abolished this lipid switch and intensified phosphorus starvation symptoms. We further identified ETHYLENE RESPONSE FACTOR 10 (StERF10) as a direct transcriptional activator of *StGSH1*. Genetic and pharmacological evidence shows that StERF10 confers LP tolerance through *StGSH1*-dependent GSH biosynthesis: BSO-mediated GSH depletion negated the LP tolerance of *StERF10*-overexpressing lines, whereas exogenous GSH rescued the hypersensitive phenotype of *StERF10*-RNAi plants. Collectively, our findings establish the StERF10-*StGSH1* regulatory module as a critical nexus linking GSH biosynthesis to lipid-mediated phosphorus usage and provide a rational target for molecular breeding of phosphorus efficient potato cultivars.

P38 Deciphering the relationships between protein persulfidation and oxidative stress in poplar exposed to elevated ozone

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Protein persulfidation, the oxidative modification of cysteine residues to persulfides, is suggested to represent the main way by which hydrogen sulfide (H_2S) exerts its biological functions (Filipovic et al., 2018). In plants, persulfidated proteins are involved in a wide range of important processes such as carbon metabolism, responses to abiotic and biotic stress, growth and development, protein translation, and autophagy (Aroca et al., 2017). However, the role of protein persulfidation in the response to oxidative stress in plants has not yet been studied. Ozone (O_3) is not only a major air pollutant and real threat to plant health and productivity, but also an excellent tool to induce oxidative stress (Noctor et al., 2016). We investigated the relationships between protein persulfidation and oxidative stress in poplar in the context of an acute O_3 stress. First, the level of persulfidated proteins was assessed in leaves of various poplar genotypes (exhibiting various sensitivity to O_3) treated or not with 250 ppb O_3 for three days. Then, the dynamic of the levels of persulfidated proteins was studied over the time of O_3 treatment and compared to the antioxidant capacity (i.e. quantifying oxidized and reduced forms of ascorbate and glutathione). Finally, we performed a proteomic analysis of the O_3-dependent persulfidome of poplar and identified several enzymes involved in the ascorbate-glutathione pathway and in lignin biosynthesis. Overall, our findings suggest that the O_3-induced oxidative stress promotes the persulfidation of specific proteins that could determine better plant tolérance to oxidative stress. Further analysis of the impact of persulfidation on protein function for some candidates will advance our understanding of ozone-dependent protein persulfidation in poplar.

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P39 Identifying potential actors promoting protein persulfidation in plants

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Protein persulfidation corresponds to the addition of an extra sulfur atom on a cysteine thiol group, leading to the formation of a persulfide group. In *Arabidopsis thaliana*, 2015 and 5214 proteins were respectively identified as persulfidated in the leaves and roots of wild-type plants grown under controlled conditions (Aroca et al., 2017; Jurado-Flores et al., 2021). These proteins are involved in a variety of biological processses such as carbon metabolism, plant responses to abiotic and biotic stresses, plant growth and development, and protein translation. In addition to hydrogen sulfide signalling, several pathways have been proposed to promote protein persulfidation. Nevertheless, the actors promoting protein persulfidation in plants remain largely unknown. To address this knowledge gap, there is a need for efficient and reliable methods to detect persulfidated proteins. Based on the previous observation that the fluorescent alkylating agent monobromobimane (mBBr) is suitable for the the purpose of conserving persulfides under biologically relevant conditions (Schilling et al., 2022), we have adapted a protocol enabling to measure protein persulfidation levels in leaf plant extracts. Various sulfurtransferases (STR1, STR2 and STR18), the cysteine desulfhydrase DES1 and the cysteine desulfurases ABA3 and NFS2 from *A. thaliana* were then assessed for their ability to promote protein persulfidation. As well as demonstrating the usefulness of the mBBr-based method for candidate screening, our findings reveal that STR1 and STR2 significantly increase the level of persulfidated proteins, whereas STR18 does not. Further comparison of the level of persulfidated proteins between wild-type and mutant plants for the most promising candidates should help identify the key catalysts of protein persulfidation in plants.

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P40 CRISPR-Based Domestication of *Tropaeolum tuberosum*: Harnessing Sulfur Metabolism for Next-Generation Crops

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This project aims to develop next-generation crops that can meet the challenges of climate change, food security, and nutrition through genome engineering. *Tropaeolum tuberosum* (mashua), a high-yielding Andean tuber with up to 17% protein, offers a nutritional profile superior to potato – including high levels of sulfur-rich amino acids (methionine, cysteine) and sulfur-containing secondary metabolites (glucosinolates). However, strong off-flavors derived from sulfur compounds and strict daylength preferences restrict its cultivation. By integrating multi-omics and transgene-free CRISPR/Cas9, I will dissect sulfur-related metabolic pathways, improve flavor by tailoring sulfur-derived metabolites, and adapt mashua for broader cultivation. This work will highlight how sulfur metabolism shapes both plant adaptation and human nutrition, and demonstrate how modern genomic tools can unlock the potential of underutilized crops.

P41 Polycomb Repressive Complex 2 regulates sulfur stress response

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Sulfur deficiency triggers an orchestrated regulation of a large number of genes [1]. A few transcription factors, including SULFUR LIMITATION 1 (SLIM1), have been identified as critical components in the sulfur deficiency response [2]. However, reverse genetic studies and biochemical analysis of these transcription factors show that they can act only on a restricted subset of sulfur-responsive genes. Therefore, the nuclear regulation of sulfur stress genes remains largely elusive.

In this study, we investigated the chromatin landscape in general nutrient stress conditions and more specifically in sulfur stress conditions in *Arabidopsis thaliana* [3]. *In silico* analysis revealed that the repressive histone mark H3K27me^3 was a critical histone modification, widely present in nutrient stress genes, including those induced by sulfur stress. We showed that not in short-term (72h or less) but in long-term (7 days or more) sulfur deficiency conditions, H3K27me^3 level dropped in the sulfur stress genes. H3K27me^3 is deposited by the Polycomb Repressive Complex 2 (PRC2), and CURLED LEAF (CLF) is the catalytic subunit of the PRC2 complex. The analysis of *clf-29* mutant in sulfur-deficient conditions revealed that the mutants were more insensitive to the stress when compared to *Col-0* controls.

In addition to its well-established developmental role, we postulate that PRC2 is a central regulator of sulfur stress. As PRC2 recruitment to specific sites requires non-coding RNA sequences, this study implicates that ncRNAs in sulfur stress should be further investigated for the nuclear regulation of sulfur deficiency genes.

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P42 Dissecting Glutathione/Glutaredoxin-Mediated Cysteinyl Redox Modifications in Plastids

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In chloroplasts abiotic stress conditions such as high light can shift cysteinyl redox steady state balance towards oxidation. This dynamic redox modification has the potential to transform localized organelle stress responses into systemic global stress response to influence plant growth and development. To counteract oxidative modifications of cysteinyl groups, chloroplast contains two major redox systems: Thioredoxin (TRX)-dependent and Glutathione/Glutaredoxin (GSH/GRX)-dependent pathways. Unlike in mitochondria and the cytosol, where these systems can compensate for each other, the plastidial GSH/GRX system is indispensable in the stroma where glutathione reductase (GR) is necessary to maintain a highly reduced GSH pool. The TRX-dependent pathway is well known for facilitating rapid adjustment of CO_2 assimilation in accordance with the photosynthetic electron transport (PET), operating thiol switches on targeting Calvin–Benson–Bassham (CBB) cycle enzymes. However, despite the evolutionary conservation of the GSH/GRX system, significant knowledge gaps remain regarding the identity of protein targets of GRX enzymes and their precise role in controlling cysteinyl steady states in chloroplast. We showed that stromal class I GRXs keep target Cys near thermodynamic equilibrium with E_{GSH}. Additionally, GR mutants with reduced stromal GR activity (*miao*) exhibit elevated and prolonged non-photochemical quenching in both ambient and high light conditions. We design a proteomics-based approach to reveal specific GRX-dependent protein targets with altered redox state or protein abundance using plastids lacking class I GRX.

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