The 12th International Plant Sulfur Workshop

July 15-18, 2022



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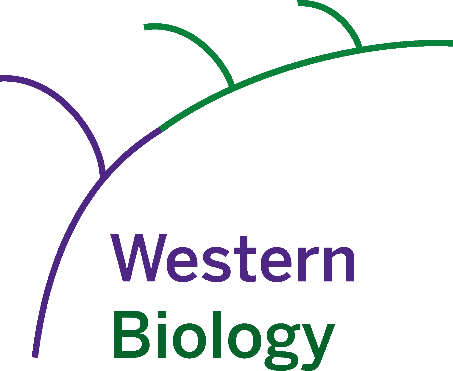






Silver





About us

|  |  |
| --- | --- |
| Organizing Committee | |
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| Mario Malagoli | Markus Wirtz |
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**Conference Schedule**

|  |  |  |
| --- | --- | --- |
| (Yellow) Presented In Person |  |  |
| (Blue) Presented Online (local time) |  |  |
| **Friday, July 15** |  |  |
| 5:30 PM | Registration | room 3110, University College |
| 6:00 PM | Get Together | room 3110, University College |
| 7:00 PM | Dinner on your own |  |
| **Saturday, July 16** |  |  |
|  |  |  |
| **Frédéric Marsolais** | **Opening Plenary Session** | **Conron Hall, University College** |
| 9:00 AM | Welcome:  Matt Davison, Dean, Faculty of Science - Frédéric Marsolais and Hideki Takahashi |  |
| 9:15 AM | Grant Maltman, Banting lecture | INSULIN 100 - BANTING HOUSE AND THE IMPORTANCE OF PLACE |
| 9:30 AM | Andrew Hanson (USA) (9:30 AM) | THE MOST EXPENSIVE SULFUR ATOM IN PLANTS AND HOW SYNTHETIC BIOLOGY CAN CUT ITS COST |
|  |  |  |
| 10:30 AM | Coffee Break |  |
|  |  |  |
| **Hideki Takahashi** | **Sulfur cycle and biotic interactions** |  |
| 11:00 AM | Eve-Lyn Hinckley (USA) (9:00 AM) | DRIVERS AND IMPLICATIONS OF THE RISE IN AGRICULTURAL SULFUR USE |
| 11:30 AM | Ryohei Thomas Nakano (Germany) (5:30 PM) | GLUCOSINOLATES MEDIATE ROOT-MICROBIOTA INTERACTIONS |
| 12:00 PM | Sorina Popescu (USA) (11:00 AM) | OSCILLATORY GENE EXPRESSION IN SYSTEMIC IMMUNITY REQUIRES REDOX-RESPONSIVE THIMET OLIGOPEPTIDASES |
| 12:15 PM | Jeffrey Waller (Canada) (1:15 PM) | EVIDENCE THAT 3-DIMETHYLSULFONIOPROPIONATE BIOSYNTHESIS IN RED MACROALGAE IS INITIATED USING A METHIONINE DEHYDROGENASE |
| 12:30 PM | Lunch |  |
|  |  |  |
| **Ann Cuypers** | **Sulfur metabolism and regulation I** |  |
| 2:00 PM | Andreas Meyer (Germany) | THE ROLE OF OXIDATIVE PROTEIN FOLDING IN THE ENDOPLASMIC RETICULUM FOR DEFENCE AGAINST REDUCTIVE STRESS AND HYPOXIC CONDITIONS |
| 2:30 PM | Luca Pedroletti (Germany) | EFFICIENT GLUTAREDOXIN S15-MEDIATED [2FE-2S] TRANSFER IS ESSENTIAL FOR ACTIVITY OF MITOCHONDRIAL LIPOYL SYNTHASE |
| 2:45 PM | Jérémy Couturier (France) (8:45 PM) | RHODANESE DOMAIN-CONTAINING SULFURTRANSFERASE FAMILY: A YET MYSTERIOUS COMPONENT OF SULFUR TRAFFICKING IN PLANTS |
| 3:15 PM | Damien Caubrière (France) (9:15 PM) | DECIPHERING THE BIOCHEMICAL RELATIONSHIPS BETWEEN CYSTEINE DESULFURASE AND SULFURTRANSFERASE PROTEIN FAMILIES IN SULFUR TRAFFICKING |
| 3:30 PM | Ruediger Hell (Germany) (9:30 PM) | A NOVEL ROLE OF CYSTEINE SYNTHESIS FOR ABA FORMATION AND STOMATA CLOSURE DURING SOIL DRYING |
| 3:45 PM | Wiebke Leemhuis (Germany) | AN UNEXPECTED FUNCTION OF THE HIGHLY CONSERVED SERAT3 ISOFORMS IN THE CYTOSOL OF ARABIDOPSIS |
| 4:00 PM | Coffee Break |  |
|  |  |  |
| **Markus Wirtz** |  |  |
| 4:30 PM | Rainer Hoefgen (Germany) | REGULATION OF SULFATE METABOLISM BY O-ACETYLSERINE |
| 4:45 PM | Anastasia Apodiakou (Germany) | FUNCTION AND REGULATION OF THE OAS CLUSTER GENES SDI1 AND SDI2 |
| 5:00 PM | José María López Ramos (Germany) | TRANSCRIPTION FACTORS SLIM1, RVE1 AND RVE8 REGULATE OAS CLUSTER GENES |
| 5:15 PM | Justyna Piotrowska (Poland) | GENERATION OF SINGLE AND MULTIPLE ARABIDOPSIS THALIANA MUTANTS IN LSU (RESPONSE TO LOW SULFUR) GENES AND THEIR PRELIMINARY ANALYSIS |
| 5:30 PM | Marcin Olszak (Poland) | ANALYSIS OF THE PROMOTER REGIONS OF LSU (RESPONSE TO LOW SULFUR) GENES IN ARABIDOPSIS THALIANA |
| 5:45 PM | Agnieszka Sirko (Poland) | SELECTIVE AUTOPHAGY CARGO RECEPTOR MODULATES SULPHUR STARVATION RESPONSE IN ARABIDOPSIS THALIANA |
|  |  |  |
| 7:00 PM | Dinner on your own |  |
|  |  |  |
| **Sunday, July 17** |  |  |
| **Stan Kopriva** | **Sulfur metabolism and regulation II** |  |
| 8:30 AM | Fang-Jie Zhao (China) (8:30 PM) | MANIPULATING SULFUR METABOLISM TO ENHANCE HEAVY METAL TOLERANCE AND IMPROVE SAFETY AND NUTRITION OF RICE |
| 9:00 AM | Rachel Amir (Israel) (4:00 PM) | HIGH LEVELS OF METHIONINE IN ARABIDOPSIS SEEDS RESULT IN HIGHER INFLUX OF NUTRIENTS FROM THE LEAVES TOWARD THE SEEDS |
| 9:15 AM | Takehiro Ito (Japan) (10:15 PM) | Γ-GLUTAMYL PEPTIDASE 1 DEGRADES GLUTATHIONE AND PARTICIPATES IN BOTH PRIMARY AND SECONDARY SULFUR METABOLISM |
| 9:30 AM | Naoko Ohkama Ohtsu (Japan) (10:30 PM) | PHYSIOLOGICAL ANALYSIS OF THE CYSTEINYLGLYCINE DEGRADING ENZYME THAT FUNCTION IN GLUTATHIONE DEGRADATION IN ARABIDOPSIS THALIANA |
| 9:45 AM | Liu Zhang (Japan) (10:45 PM) | DISRUPTION OF SULFUR DEFICIENCY-INDUCED GLUCOSINOLATES CATABOLISM AFFECTS GLUCOSNILATES DISTRIBUTION IN MATURE PLANTS |
| 10:00 AM | Coffee Break - Virtual Posters from Asia |  |
|  |  | Kang-Di Hu, Xiang-Jun Peng, Yu-Qi Zhao, Lin Ma, Si-Yue Wang, Gai-Fang Yao, Hua Zhang (10:00 PM) CYSTEINE DESULFHYDRASE LCD1 INTERACTS WITH IMPORTIN Α3 AND REGULATES TOMATO FRUIT RIPENING AND SENESCENCE IN TOMATO |
|  |  | H.T Nguyen, A. Suyama, A. Maruyama-Nakashita (11:00 PM) ANTHOCYANIN PRODUCTION IN ARABIDOPSIS ROOTS USING THE DOWNSTREAM REGION OF SULFATE TRANSPORTER SULTR2;1 |
|  |  |  |
| **Hideki Takahashi** |  |  |
| 10:30 AM | Yan Xiong (China) (10:30 PM) | SULFATE-TOR SIGNALLING CONTROLS TRANSCRIPTIONAL REPROGRAMMING FOR SHOOT APEX ACTIVATION |
| 11:00 AM | Xinyuan Huang (China) (11:00 PM) | LOCAL AND SYSTEMIC RESPONSE TO HETEROGENEOUS SULFATE RESUPPLY AFTER SULFUR DEFICIENCY IN RICE |
| 11:15 AM | Karine Gallardo (France) | A HOLISTIC OVERVIEW OF THE IMPACT OF SULFUR DEFICIENCY IN PEA FACING WATER STRESS |
| 11:30 AM | Fanélie Bachelet (France) (5:30 PM) | ROLE OF VACUOLAR SULFATE IN NUTRITIONAL QUALITY OF PEA SEEDS |
| 11:45 AM | Anna Wawrzynska (Poland) (5:45 PM) | SUGAR SIGNALING IS AFFECTED BY SLIM1 DURING SULFUR DEFICIENCY IN ARABIDOPSIS THALIANA. |
| 12:00 PM | Lunch & Posters from Europe |  |
|  |  | V. MANTA, E. KAROUSIS, G. STYLIANIDIS, A.TZANAKI, D. DIMITRIADI, S.N. CHORIANOPOULOU, D.L. BOURANIS COMBINED BIOFORTIFICATION OF BROCCOLI HEADS WITH SELENIUM, CYSTEINE, AND/OR METHIONINE: A POTENTIAL APPROACH TO OVERCOME THE ANTAGONISTIC RELATIONSHIP BETWEEN SULFUR AND SELENIUM? |
|  |  | Y.E. VENTOURIS, S. KARAMANOLIS, V.G. MAGOULIANITIS, K.-G. STAMELOS, G. STYLIANIDIS, D.L. BOURANIS, S.N. CHORIANOPOULOU (6:00 PM) EFFECT OF IRON AND GIBBERELLIN INTERPLAY ON SULFUR HOMEOSTASIS OF MAIZE |
|  |  |  |
| **Karine Gallardo** | **Sulfur metabolism and regulation III** |  |
| 2:00 PM | Markus Wirtz (Germany) | NOVEL LINKS BETWEEN ENERGY SENSOR KINASES AND THE ASSIMILATORY SULFUR REDUCTION PATHWAY |
| 2:30 PM | Barbara Moffatt (Canada) | THE EFFECTS OF REDUCED METHYLTHIOADENOSINE RECYCLING ARE RESPONSIVE TO INCREASED SPERMIDINE SUPPLY |
| 3:00 PM | Saeer Adeel (Canada) | A GENETIC APPROACH TO DECIPHER THE IMPACT OF METHYTHIOADENOSINE ACCUMULATION ON PLANT DEVELOPMENT AND SULFUR METABOLISM |
| 3:15 PM | Frédéric Marsolais (Canada) | THE CYSTEINE-RICH 11S GLOBULIN LEGUMIN FROM COMMON BEAN CONTAINS A PEPTIDE RESISTANT TO CLEAVAGE BY PEPSIN (pH ≥ 2) AND CHYMOTRYPSIN |
| 3:30 PM | Zixuan Lu (Canada) | THE BIOSYNTHESIS OF NON-PROTEIN SULPHUR AMINO ACID IN SEED OF COMMON BEAN |
| 3:45 PM | Coffee Break |  |
|  |  |  |
| **Rainer Hoefgen** |  |  |
| 4:15 PM | Dimitris Bouranis (Greece) | TOWARDS INTEGRATED SULFUR-BASED BIOSTIMULATION AND AGRONOMIC BIOFORTIFICATION OF OLIVE TREE PRODUCTION |
| 4:30 PM | Spencer Matt (Canada) | EFFECTS OF EXCESS SULFUR ON CADMIUM UPTAKE AND TRANSLOCATION IN SOYBEAN |
| 4:45 PM | Baoluo Ma (Canada) | INTERACTIVE EFFECT OF NITROGEN AND SULFUR SUPPLY ON YIELD AND RELATED TRAITS OF CANOLA |
| 5:00 PM | David Mendoza-Cózatl (USA) (4:00 PM) | UNDERSTANDING THE MOLECULAR MECHANISMS MEDIATING THE CROSSTALK BETWEEN IRON AND SULFUR NETWORKS IN PLANTS |
| 5:30 PM | Landon Swartz (USA) | TRACKING DYNAMIC CHANGES OF LEAVES IN RESPONSE TO NUTRIENT AVAILABILITY USING AN OPEN-SOURCE CLOUD-BASED PHENOTYPING SYSTEM (OPEN LEAF) |
| 5:45 PM | Ann Cuypers (Belgium) | SULFUR IN PLANT RESPONSES TO CADMIUM STRESS: FROM SIGNALING TO ACCLIMATION |
| 6:00 PM | Closing: Frédéric Marsolais and Hideki Takahashi |  |
|  |  |  |
| 7:00 PM | Conference Dinner | Thames Hall Atrium |
|  |  |  |
| **Monday, July 18** |  |  |
|  | Niagara Falls Excursion | Bus Trip to Wayne Gretzky Estates in the scenic destination of Niagara-on-the-Lake.  Departing Ontario Hall at 8:35am  Arriving to Wayne Gretzky Estates at 11:30am for a 35 minute Wine, Whisky and Beer tasting. Lunch seating is reserved for us at the Whisky Bar Patio at the Wayne Gretzky Estates starting at 12:15pm. Lunch cost is on own.  The bus will depart for Niagara Falls at 1:30pm. Explore Niagara Falls from 2pm to 4:30pm. The bus will arrive back in London at Ontario Hall at roughly 7:00pm  https://www.niagarafallstourism.com/play |

Abstracts

**THE MOST EXPENSIVE SULFUR ATOM IN PLANTS AND HOW SYNTHETIC BIOLOGY CAN CUT ITS COST**

A.D. HANSON\*, K.R. VAN GELDER\*, J.D. GARCÍA-GARCÍA\*\*

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The sulfur atom in the thiazole ring of thiamin costs >2,800 ATP to produce. This because the THI4 thiazole synthase that forms the ring is a suicide enzyme that uses a Cys residue in its active site as sulfur donor, causing inactivation and forcing complete degradation and resynthesis of the THI4 after one reaction cycle (Hanson et al., 2018; Joshi et al., 2020). This makes THI4 the fastest-turnover plant enzyme (Li et al., 2017) and one of the most energetically expensive to operate, with a cost equivalent to 2-4% of biomass yield (Hanson et al., 2018). Swapping a crop’s native suicide THI4 for a thiazole synthase that mediates multiple reaction cycles could thus potentially increase biomass by 2-4%, making this a synthetic biology strategy for yield improvement (Dastmalchi, 2022).

A straightforward way to execute this strategy is to exchange the plant suicide THI4 for a different type of THI4 that uses sulfide as sulfur donor and catalyzes multiple reaction cycles (Sun et al., 2019). However, there are barriers to doing this. Sulfide-dependent THI4s are unlikely to function optimally in plants because they basically come from prokaryotes from habitats that are (i) anoxic or deeply hypoxic, (ii) sulfide-rich, and (iii) in certain cases extremely hot (≥60°C) (Joshi et al., 2021). The challenge is thus to repurpose sulfide-dependent THI4s to work well at 21% oxygen (vs. <1%), low-µM intracellular sulfide levels (vs. high-µM to mM), and moderate temperatures.

Continuous directed evolution is an ideal approach for meeting this challenge due to its unmatched capacity to achieve scale and depth in evolutionary searches (Rix and Liu, 2021). In continuous directed evolution, the target gene undergoes hypermutation *in vivo*, target protein activity is coupled to host cell growth, and improved variants are obtained simply by selecting for faster growth. We chose the yeast OrthoRep system for its capacity to mutate a target gene ~100,000-fold faster than the natural mutation rate and its durability (Ravikumar et al., 2018). We coupled activity of the target sulfide-dependent THI4 to yeast growth by using a Δ*thi4* deletant host strain that also carried a Δ*met15* deletion to raise internal sulfide level (Brachmann et al., 1998), and selected for growth with minimal or no thiamin supplementation (García-García et al., 2022). We tried to evolve THI4s from the mesophiles *Mucinivorans hirudinis* (from leech gut, MhTHI4) and *Saccharicrinis fermentans* (from marine mud, SfTHI4), and the hyperthermophile *Thermovibrio ammonificans* (from a hydrothermal vent, TaTHI4). Improved variants with one or two mutations each were obtained for MhTHI4 and SfTHI4, but not TaTH4. At least two of the beneficial mutations could be rationalized as likely adaptations to high oxygen level. The improved variants conferred growth rates similar to yeast native THI4 controls, indicating arrival at or near the upper limit of the selection window. We are now running further directed evolution campaigns that start from the above improved variants and expand the selection window by dialing down the THI4 expression level or adding a thiazole synthase inhibitor to the medium. We ultimately plan to introduce the evolved THI4 genes into plants via gene editing or, if this fails, by transgenesis.

More generally, this research prototypes a crop breeding strategy that uses continuous directed evolution in a microbe to quickly improve a gene from a crop or another organism, then returns or introduces the gene to the crop by gene editing, which the USA and many other countries treat as equivalent to conventional plant breeding.

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DRIVERS AND IMPLICATIONS OF THE RISE IN AGRICULTURAL SULFUR USE

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Sulfur (S) is a critical element for plant growth. As atmospheric S deposition has declined in response to air quality regulation in the United States and Europe, there has been an increase in S fertilizer applications reported in many major crop systems. In addition, intensification of agriculture has driven increased S inputs for other uses: as a pesticide, regulator of soil pH, and soil conditioner. In this talk, I weave together evidence from S mass balance calculations, long-term trend analysis, and process-based biogeochemistry to describe how and why agricultural S use is changing, as well as the biogeochemical and ecological consequences. First, I present trends in S input and export in three U.S. agricultural regions with different histories of agricultural S inputs and atmospheric S deposition: winegrapes in California where S is used as a fungicide, corn and soy in the Ohio River Valley where it is used as fertilizer, and sugarcane in Florida where S is used to regulate soil pH and improve phosphorus availability and compare these with a forested reference area in the Northeast. Second, I focus on the Midwestern U.S. and present the first compiled dataset of S fertilizer sales in the region as a proxy for S inputs from 1985-2015. An analysis of watershed inputs/outputs in case study areas demonstrates that surface water sulfate export has declined about 2-5% per year over the past 30 years in areas of the northeastern U.S. historically impacted by acid rain and where crop systems are dispersed. Conversely, stream water sulfate concentrations have increased or remained elevated in the three large agricultural case study areas. The 30-year trend in S fertilizer sales in the Midwestern U.S. shows that as corn and soy have expanded and yields have increased, S fertilizer sales have risen significantly. This increase is commensurate with declines in historically high atmospheric S deposition across the region, from ~5 to 1 kg S ha-1 yr-1 and far outpaces the rate of change in other major fertilizers over the same period; S has increased nearly 300% since 1985, while N, P, and potassium have increased minimally or remained flat. With sustained agricultural productivity and continued declines in atmospheric S deposition, farmers will continue increasing the use of S products to meet crop demand or manage soils. Targeted S inputs to large, regional agricultural systems may have significant consequences for soil health and adjacent aquatic ecosystems, as demonstrated by measurements of sulfate reduction and production of methylmercury, a neurotoxin that threatens wildlife and people, downstream of California agricultural areas where S is applied. These multiple lines of evidence suggest that regulatory measures and further research on sustainable S management must be a near-term priority.

**GLUCOSINOLATES MEDIATE ROOT-MICROBIOTA INTERACTIONS**

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*\*\*\* Max Planck Institute for Plant Breeding Research, Cologne, Germany*

Plants are chronically exposed to a large diversity of microbes, a part of which successfully colonize plant tissues to form a plant-associated microbial community called the plant microbiota. Despite the known impact of these microbes on plant physiology, the molecular mechanisms by which plants and the associated microbiota communicate with each other remain elusive. It has been reported that different plant species accommodate different compositions of root microbial communities (Wippel and Tao et al., 2021), which led us to hypothesize that plant lineage-specific secondary metabolites play a role in transferring signals from plants to microbes.

Plants in Brassicales order accumulate a large amount of sulfur-containing secondary metabolites, called the glucosinolates, which are conjugates of an aglycon and glucose via an S-glycosidic bond. Intact glucosinolates are biologically inactive, while the free aglycon released by hydrolysis by a specific class of -glucosidases ("myrosinases") is immediately converted into highly reactive molecules, such as isothiocyanates. These intermediate and/or terminal products of glucosinolate activation have been shown to play a crucial role in defense against herbivorous insects (Barth and Jander, 2006) and pathogenic microbes (Bednarek et al., 2009; Clay et al., 2009), as well as in the proper management of interactions with beneficial microbes (Lahrmann et al., 2015; Hiruma et al., 2016). In contrast, to which extent glucosinolates are relevant beyond pathogenic and beneficial interactions, i.e. for interactions with commensal microbes that constitute plant microbiota, remains unknown.

Here, we investigated the role of Trp-derived indole glucosinolates (IGs) in the assembly of microbiota associated with *Arabidopsis thaliana* roots. In addition to the mutants impaired in IG biosynthesis, we analyzed mutants impaired in PYK10, a recently identified myrosinase that is among the most abundant proteins in *A. thaliana* roots (Nakano et al., 2017). PYK10 and its closest homolog BGLU21 localize in an endoplasmic reticulum (ER) body (Matsushima et al., 2003), an ER-derived membrane structure that is developed explicitly in plants of Brassicaceae and related families (Nakano and Yamada et al., 2014). We have shown that ER body development is transcriptionally corelated with the glucosinolate biosynthetic pathways (Nakano et al., 2017), pointing to their functional coordination. By growing these mutants and the wild-type Col-0 in natural soils, we showed that these IGs and ER body pathways play a role in the bacterial and fungal community assembly in rhizoplane (root surface) and endosphere (root interior), respectively. By treating natural soils or bacterial synthetic communities (an *in vitro* mixture of individually grown bacterial strains) with root exudates collected from these plants, we demonstrated that root-secreted compounds are important for IG- and ER body-mediated bacterial community assembly. We also found that endophytic fungi isolated from healthy Col-0 plants inhibited the growth of these mutants more severely than of Col-0 plants, pointing to the role of IGs and ER bodies in fungal accommodation. Overall, our results illustrate a microbiota assembly process that is mediated by root-secreted compounds, plausibly including sulfur-containing metabolites, which delineates a lineage-specific innovation of plant metabolism and an intracellular component that is relevant under natural conditions.

**OSCILLATORY GENE EXPRESSION IN SYSTEMIC IMMUNITY REQUIRES REDOX-RESPONSIVE THIMET OLIGOPEPTIDASES**

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In plants, pathogen recognition activates a local immune response in the tissues surrounding the infection site and systemic immunity in distal pathogen-free tissues. Local and systemic immune reactions allow the plant to prioritize cellular processes that inhibit pathogen multiplication and spread and, simultaneously, to build an immunological memory, which protects the plant from further infections. Signal transduction for systemic defense begins at the primary pathogen infection site with the activation of local immune responses. A type of local immunity, the effector-triggered immunity (ETI), is initiated when immune receptors recognize distinct pathogen effector proteins secreted into the plant. Activated immune receptors trigger complex signaling networks that converge on defined genetic programs regulating the expression of hundreds of genes for hormone synthesis, metabolic antimicrobial defense, and programmed cell death (PCD). Long-distance signals produced in the pathogen-infected tissue are transported systemically to pathogen-free tissues, enabling a defense primed state characterized by transcriptional and metabolic adjustments such as accumulating dormant signaling components. This primed state leads to systemic acquired resistance (SAR), enabling a faster and more robust immune response to subsequent infections from a broad spectrum of pathogens.

The redox poise of the plant cell is centrally involved in the activation of the genetic and metabolic programs of both local and systemic immunity. Following pathogen recognition, a local oxidative burst occurs, characterized by increased production and accumulation of reactive oxygen species (ROS) catalyzed by enzymes located at the cell periphery and organelles. Systemic oxidative micro-bursts follow the local oxidative bursts and are required for SAR. The impact of the cellular redox potential on plant immunity is substantial. Profiling redox-mediated transcription and studies of mutants defective in redox homeostasis provided abundant evidence for incorporating ROS production and scavenging systems into immune responses. Whereas the genetic program activated at the site of pathogen recognition has been intensely scrutinized, fewer studies have tackled the program regulating systemic immunity and the associated redox-modulated processes.

Recent analyses of the redox-regulated oligopeptidases TOP1 and TOP2 indicated a role for controlled proteolysis in local immune processes such as PCD, pathogen-triggered oxidative burst, and reversible proteome oxidation. TOPs enzymatic activities were enhanced locally and systemically in wild-type Arabidopsis undergoing SAR but not mutants defective in redox homeostasis. Nevertheless, substantial gaps remain in our knowledge regarding how the localized ETI program shifts to systemic immune priming and SAR. The overarching structure of the redox-regulated network during the SAR is yet to be elucidated, and an understanding of the temporal dynamics of SAR-associated gene activity in relation to the cellular redox state is lacking.

Here we have addressed these issues by analyzing the genetic interaction of *TOP1* and *TOP2*. Strikingly, the SAR-null phenotype of the *top2* mutant fully recovered in *top1top2* that lacks both *TOP1* and *TOP2*, indicating that deficiency of T*OP1* suppresses the t*op2* SAR phenotype (Figure 1). We characterized the suppressor effect and propose that antagonistic signals relayed through *TOPs* are integrated to control redox signaling and temporal dynamics of the SAR transcriptional program. Among the most critical points to emerge from this study is the observation that *TOPs* are essential for sustaining a wave-like temporal pattern of redox-sensitive transcript accumulation**.** Our findings are, in principle, consistent with a mechanism whereby information necessary for organizing a systemic immune response is encoded in the amplitude and temporal patterns of gene expression.

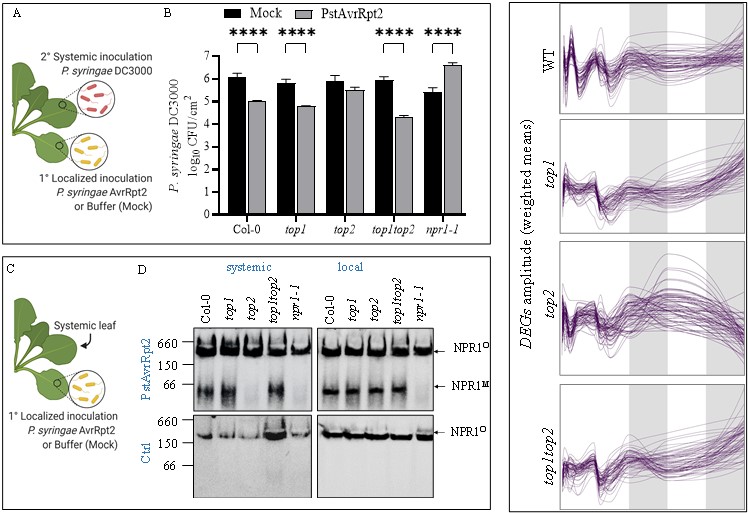


Figure 2. The expression of numerous SAR genes oscillates. Synchronized SAR transcriptome requires inputs from *TOPs* to maintain the amplitude and period of oscillations.

Figure 1. SAR is abolished in *top2* and rescued in *top1top2* background.

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**EVIDENCE THAT 3-DIMETHYLSULFONIOPROPIONATE BIOSYNTHESIS IN RED MICROALGAE IS INITIATED USING A METHIONINE DEHYDROGENASE**

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Dimethylsulfoniopropionate (DMSP) is a zwitterionic sulfonium compound produced by marine organisms, serving roles such osmoprotection, cryoprotection, and perhpas a key role in the global biogeochemical sulfur cycle. The canonical marine algal DMSP biosynthesis pathway begins with the deamination of methionine into 4-methylthio-2-oxobutyrate (MTOB) via a 2-oxoglutarate-depenent aminotransferase. DMSP’s gaseous breakdown product dimethylsulfide (DMS) and DMSP itself were first discovered in the red macroalgal species *Vertebrata fastigiata* (formerly *Polysiphonia fastigiata*) nearly a century ago. Subsequently, the DMSP pathway was elucidated using metabolite tracer and enzymological studies but largely in Chlorophyte green macroalgal *Ulva* species (formerly *Enteromorpha*) with some evidence for it in prymnesiophyte, diatoms, and prasinophytes, and some marine bacteria. Following the discovery of all four genes responsible for DMSP synthesis in *Ulva mutabilis,* comparative genomic techniques were used to investigate the presence of homologues to the *U. mutabilis* enzymes in other algal species including *V. japonica* and related Rhodophytes. Comparative genomic methods helped us discover a putative methionine dehydrogenase (MetDH) in *V. japonica* which was then cloned, expressed in bacteria, and characterized. This putative *V. japonica* methionine dehydrogenase (*Vj*MetDH) would be the first amino acid dehydrogenase to show naturally high specificity for methionine. *Vj*MetDH kinetics parameters were also found and compared to those of other Met-specific deaminating enzymes. *Vj*MetDH had a lower *K*M for Met than its counterparts at 34 µM and similar NAD+ affinity at 650 µM to related amino acid dehydrogenases. Incubating *Vj*MetDH with additives DTT, ADP, and divalent ions all activated the enzyme, indicating potential roles of thiol groups, in ATP biosynthesis, and *Vj*MetDH stability, respectively. The collected data provides insight into the novel *Vj*MetDH while advancing our current understanding of DMSP biosynthesis pathways.

**The role of oxidative protein folding in the endoplasmic reticulum for defence against reductive stress and hypoxic conditions**

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Formation of structural disulfides is essential for many ER-resident proteins and for proteins passing the ER on the way to their final destination. De novo disulfide formation by ER oxidoreductins (EROs) is followed by transfer of disulfides to nascent peptides through a cascade of thiol-disulfide exchange reactions involving several disulfides in ERO and protein disulfide isomerases (PDIs). Despite largely homologous sequences, the two isoforms ERO1 and ERO2 encoded in the genome of *Arabidopsis thaliana* are present in distinct redox states indicating that they might be regulated differently and possess different activities. Nevertheless, both proteins as well as coordinated action of EROs and PDIs are required for oxidative protein folding. The net transfer of two electrons to molecular oxygen by EROs leads to the formation of stoichiometric amounts of H2O2 as a potentially toxic by-product that demands appropriate detoxification mechanisms. A candidate for local detoxification of H2O2 in the secretory pathway is glutathione peroxidase-like 3 (GPXL3), which resides as a type II membrane protein in the ER. GPXL3 in conjunction with PDIs may increase the efficiency of the oxidative folding machinery by a factor of two, if all generated H2O2 can be recycled via this route. Non-native and thermodynamically less stable protein disulfides generated during oxidative protein folding are prone to non-catalyzed, thermodynamically driven attack of GSH. Glutathionylated peptides may re-enter the folding pathway after removal of the glutathione moiety. Deglutathionylation of proteins can be mediated by two dithiol glutaredoxins (GRXs), which are also present in the secretory pathway as type II membrane proteins.

Insufficient disulfide formation may lead to the accumulation of unfolded proteins and ultimately an unfolded protein response (UPR). Such situations may occur under reductive stress situations originating from metabolic imbalances or during hypoxia resulting from flooding and thus limited oxygen supply. Strict dependence of ERO activity on molecular oxygen as the final electron acceptor implies that oxidative protein folding and other ER processes are severely compromised under hypoxia. Accordingly, viable *ero1 ero2* double mutants are highly sensitive to reductive stress and hypoxia. To elucidate the specific redox dynamics in the ER *in vivo*, we expressed the glutathione redox potential (*E*GSH) sensor Grx1-roGFP2iL-HDEL with a midpoint potential of -240 mV in the ER of Arabidopsis plants. We found *E*GSH values of -241 mV in wild-type plants, which is less oxidizing than previously estimated. In the *ero1 ero2* mutants, luminal *E*GSH was reduced further to -253 mV. Recovery to reductive ER stress induced by dithiothreitol, was delayed in *ero1 ero2*. The characteristic signature of *E*GSH dynamics in the ER lumen triggered by hypoxia was affected in *ero1 ero2* reflecting a disrupted balance of reductive and oxidizing inputs, including nascent polypeptides and glutathione entry. Taken together, this reveals a central role of EROs as major redox integrators to promote luminal redox homeostasis. The experimental setup for dynamic redox studies in the ER of living plants establishes a technological platform that enables further dissection of ER redox dynamics in future work.

**EFFICIENT GLUTAREDOXIN S15-MEDIATED [2FE-2S] TRANSFER IS ESSENTIAL FOR ACTIVITY OF MITOCHONDRIAL LIPOYL SYNTHASE**

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Iron-sulfur clusters (ISCs) are crucial cofactors, especially in mitochondria, where they are essential for some enzymes in the TCA cycle, for electron transfer in the respiratory electron transport chain and the synthesis of the cofactors biotin and lipoic acid. ISCs are extremely unstable and thus need a chaperoning protein structure to prevent their disassembly. In mitochondria, protected transfer of [2Fe-2S] is mediated by class II glutaredoxins (GRXs). The only GRX present in plant mitochondria is GRXS15, which takes an essential role in the assembly of ISC-dependent proteins. The indispensable role of GRXS15 is supported by the fact that *grxs15* null mutants are embryo-lethal. Physiological studies with knockdown mutants and null mutants complemented with a less active GRXS15 K83A variant are viable but show different degrees of dwarfism. All these mutants are characterized by reduced activity of lipoic acid (LA)-dependent enzymes. Given that mitochondria contain 26 ISC-dependent enzymes and electron-transfer proteins, this finding is surprising and requires further elucidation of the function of GRXS15 in LA biosynthesis.

In plant mitochondria, the last step of LA biosynthesis is catalyzed by lipoyl synthase (LIP1). This enzyme contains one [4Fe-4S] buried in its core and a second auxiliary [4Fe-4S], which is sacrificed during each catalytic cycle for the provision of two sulfur atoms to the n-octanoyl precursor residing on lipoyl carrier proteins that constitute one subunit of LA-dependent dehydrogenase complexes. Stoichiometric destruction of one [4Fe-4S] cluster for each LA residue implies increased demand for either new [4Fe-4S] or repair of the fragmented cluster and thus two different possible processes in which GRXS15 could potentially be involved. Interestingly, LIP1 with an estimate of less than 100 copies per mitochondrion is far less abundant than most other [4Fe-4S] proteins. Based on these observations, we hypothesized that LIP1 is the first protein to experience a shortage of cluster supply if the capacity of the upstream transfer system is restricted.

Here, we demonstrate that diminished photorespiration under high atmospheric CO2 suppresses the dwarf phenotype of *grxs15* mutants and causes re-adjustment of the metabolite profile by overcoming the low activity of the glycine dehydrogenase complex as one of the four mitochondrial LA-dependent enzyme complexes. Furthermore, we show that deletion of the high-abundant [4Fe-4S]-dependent aconitase 3 in viable *grxs15* mutants allows the redirection of [4Fe-4S] to LIP1 and thus partially rescues the dwarf phenotype. Finally, overexpression of LIP1 in *grxs15* null mutants complemented with GRXS15 K83A generates a sink for [4Fe-4S] and completely suppresses the severe dwarfism of the respective mutants*.* Concomitantly, the accumulation of all metabolites synthesized by LA-dependent enzymes is prevented.

However, we also found that overexpression of LIP1 in wild-type plants and in *grxs15* knockdown lines is deleterious with a clear gene dosage effect, resulting in curly leaves, delay in flowering and accumulation of several metabolites, especially cysteine. We hypothesized that due to the overexpression, increased LIP1 activity leads to more [4Fe-4S] destruction with collateral sulfide accumulation in mitochondria. To avoid inhibition of cytochrome C oxidase, the sulfide is partially detoxified by the OASTLC-SERAT2;2 complex in the mitochondrial matrix, which leads to accumulation of cysteine. Here, we demonstrate that overexpression of LIP1 in the null mutants *oastlC and serat2;2* as well as the respective double mutant indeed leads to a more severe phenotype than overexpression in wild-type plants.

Taken together, our data demonstrate that the availability of ISCs in mitochondria relies on GRXS15 and that LIP1 is the most sensitive enzyme experiencing deficiency if there is a shortfall in ISC supply. Genetically altered ISC distribution can ensure sufficient ISC supply for LIP1. The presented data allow drawing a refined model for the mitochondrial ISC transfer machinery and highlight deleterious consequences of LIP1 hyperactivity, which may lead to toxic effects due to accumulation of sulfide.

**Rhodanese domain-containing sulfurtransferase family: a yet mysterious component of sulfur trafficking in plants**

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The biosynthesis of many sulfur-containing biomolecules (iron-sulfur cluster, biotin, thiamine, lipoic acid, molybdopterin and sulfur-containing bases in tRNA) depends on cysteine as a sulfur source. Cysteine desulfurase (CD) and rhodanese (Rhd) domain-containing protein families participate in the trafficking of sulfur for various metabolic pathways in bacteria and human. However, their connection is not yet described in plants. Sulfurtransferases (STRs) consist of one or multiple Rhd domains eventually fused to other protein domains and likely act as carrier proteins required for sulfur trafficking. Most STRs catalyze the transfer of a sulfur atom from sulfur donors to nucleophilic sulfur acceptors by forming themselves protein-persulfides as intermediates on a reactive cysteine. A comparative analysis of 25 genomes of photosynthetic organisms has provided a detailed classification and information about evolutionary features of STRs pointing to the expansion of this family in higher plants. The STR family in higher plants is composed on average of 20 members that are quite divergent considering their primary sequence, protein domain organization and subcellular localization. Furthermore, their physiological function remains unclear for most of them. To decipher the molecular mechanisms of the sulfur delivery and trafficking systems in plants, we have investigated the biochemical properties of some specific STR isoforms of *Arabidopsis thaliana*, their connection with CDs but also their relationships with thioredoxin and glutathione/glutaredoxin reducing systems.

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**Deciphering the biochemical relationships between cysteine desulfurase and sulfurtransferase protein families in sulfur trafficking**

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In all living organisms, sulfur represents an essential element and is incorporated in key molecules such as sulfolipids, thionucleosides, vitamins (thiamine, biotin, lipoic acid) and cofactors such as iron-sulfur clusters or molybdenum cofactors (Mueller, 2006). Cysteine serves as the sulfur source for many sulfur-containing cofactors or molecules in both bacterial and eukaryotic systems (Mueller, 2006). These biosyntheses occur via complex multistep processes that are yet to be completely understood. The first hallmark step of sulfur extraction is catalyzed by cysteine desulfurases (CDs), leading to the formation of a persulfide bond on a catalytic cysteine. The subsequent trafficking of persulfides, so-called transpersulfidation reactions, implies the existence of sulfur carrier proteins, notably of the sulfurtransferase (STR) family. Most STRs catalyze the transfer of a sulfur atom from sulfur donors to nucleophilic sulfur acceptors by forming themselves protein-persulfides as intermediates on a reactive catalytic cysteine. Several examples of the involvement of CD/STR couples in the synthesis pathways of sulfur-containing molecules already exist in bacteria and human (Lauhon and Kambampati, 2000; Dahl et al., 2011) but not in plants despite they possess both CD and STRs isoforms in different organelles (Selles et al., 2019; Moseler et al., 2020). Here, we investigate the biochemical relationships between two cytosolic proteins from *Arabidopsis thaliana*, the CD isoform ABA3 and STR18 and compare these biochemical features to those of a natural CD-STR fusion protein from the bacterium *Pseudorhodoferax sp.* (Selles et al., 2022)*.*

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**A NOVEL ROLE OF CYSTEINE SYNTHESIS FOR ABA FORMATION AND STOMATA CLOSURE DURING SOIL DRYING**

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Water is the most limiting factor for plant growth and agricultural productivity, becoming even more important in a world of climate change. Plants have evolved multiple sensing and signalling strategies to respond to water deficit in the soil or atmosphere. Closure of stomata is is probably the fastest and most regulated of these strategies. Absisic acid (ABA) is the canonical trigger for induction of stomata closure. When plants close stomata in response to root water deficit ABA is transported form root to shoot and believed to be the first of several signals leading to stomata closure. It is now well established that soil-drying induces root-to-shoot sulfate transport via the xylem even before ABA transport (Ernst et al., 2010). In poplar, this soil-drying triggered sulfate transport is facilitated by differential expression of diverse sulfate transporters in the vasculature (Malcheska et al., 2017).

There is increasing evidence for a physiologically relevant signaling pathway that underlies sulfate-induced stomatal closure in *Arabidopsis thaliana*. We uncovered that sulfate activates NADPH oxidases to produce reactive oxygen species (ROS) in the guard cells. This ROS induction is essential for sulfate-induced stomata closure. In line with the function of ROS as the second messenger of (ABA) signaling, sulfate does not induce ROS in the ABA-synthesis mutant, *aba3-1*, and sulfate-induced ROS is ineffective for closing stomata in the ABA-insensitive mutant *abi2-1* and a SLOW ANION CHANNEL1 loss-of-function mutant. External application of sulfate results in the accumulation of ABA in the cytosol of guard cells as shown by applying the ABA FRET sensor ABAleon2.1, the ABA signaling reporter ProRAB18:GFP, and quantification of ABA marker genes (Batool et al., 2018). Furthermore, when petioles of the nearly ABA deficient mutant ABA3 that had been complemented with a construct expressing ABA3 under the control of a stomata specific promoter (Bauer et al., 2013) are fed with sulfate the stomata close, providing evidence for a stoma cell-type specific process (Batool et al. 2018). Consequently, sulfate-induced stomata closure requires the canonical ABA signal transduction machinery in stoma cells.

In addition to the identification of ABA signal transduction components (Rajab et al., 2019) we discovered the metabolic components that are indispensable for sulfate-triggered stomata closure. The sulfurtransferase ABA3 requires Cys as the sulfur donor for the activation of the enzyme catalysing the last step of ABA biosynthesis, abscisic aldehyde oxidase AAO3 (Cao et al., 2014). This finding points to cysteine as trigger for ABA production in guard cells. This hypothesis is corroborated by demonstrating that reductive assimilation of sulfate into Cys is essential for sulfate-induced stomatal closure (Batool et al., 2018). Since sulfate reduction exclusively occurs in plastids, depleting plastid-localized sulfate transporters impairs sulfate-induced stomata closure (Chen et al., 2019). Evidence for the physiological significance of these processes comes from mutants with depleted Cys synthesis that are sensitive to soil drying due to enhanced water loss. Indeed, mutants depleted in plastid Cys synthesis are sensitive to soil drying. We conclude from these studies that sulfate is incorporated into Cys and tunes ABA biosynthesis in stomata towards stomatal closure, thus contributing to the physiological water limitation response. The particular importance of subcellular compartmentalization of cysteine synthesis and chloroplast components for triggering ABA biosynthesis in stomata will be addressed.

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**AN UNEXPECTED FUNCTION OF THE HIGHLY CONSERVED SERAT3 ISOFORMS IN THE CYTOSOL OF ARABIDOPSIS**

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Cysteine biosynthesis takes place in all subcellular compartments capable of protein translation and is catalyzed by the consecutively acting enzymes: serine acetyltransferase (SERAT) and *O*-acetylserine(thiol)lyase (OAS-TL). In Arabidopsis, both enzymes are encoded by multi-gene families whose members are distributed in the cytosol, mitochondria, and plastids. Formation of the carbon nitrogen-containing precursor, *O*-acetylserine (OAS) by SERAT limits cysteine synthesis, which is achieved by replacing the activated acetyl-residue in OAS with sulfide by OAS-TL. The cytosolic SERAT1;1 and the organelle-localized SERAT2;1 and SERAT2;2 interact via a conserved C-terminal tail with OAS-TLs in a bi-enzyme protein complex called cysteine synthase complex (CSC). Since the C-terminal tails of SERATs bind into the active site of OAS-TLs (Francois et al., 2006), complex-associated OAS-TL is inactive, and the association of the CSC is controlled by the OAS-TL substrates, OAS and sulfide. Formation of the CSC activates SERATs of group 2 (Droux et al., 1998; Wirtz et al., 2012), strongly suggesting that CSC formation regulates the SERAT activity in response to the available sulfide supply.

Arabidopsis possesses two additional cytosolic SERAT isoforms (SERAT3;1 and SERAT 3;2), which cannot interact with OAS-TL and display much lower specific SERAT activities than the major SERATs of groups 1 and 2 (Kawashima et al., 2005). The presence of at least one SERAT of group 3 appears to be conserved in vascular plants. The CSC-associating thee major SERATs contribute more than 90% of total SERAT activity in Arabidopsis. Nonetheless, the presence of a single SERAT3 in the quadruple SERAT mutants Q3;1 and Q3;2 or both SERAT3 isoforms in the *serat tko* mutant is sufficient for plant survival but results in decreased growth. In contrast, the remaining activity of SERAT1;1 in the Q1;1 mutant allows for wild-type like growth under non-stressed conditions (Watanabe et al., 2018), strongly suggesting that SERAT1;1 is the major SERAT in the cytosol. Explicitly transcription of *SERAT3* genes is induced upon sulfur starvation, while the major *SERATs* and *OAS-TLs* are not transcriptionally regulated in response to sulfur supply.

In this study, we addressed the biological function of SERAT3 for cysteine biosynthesis by testing their physical interaction with cytosolic SERAT1;1 and OAS-TL A. Surprisingly, we found that both SERAT3 isoforms stably interact with SERAT1;1 *in planta*. We addressed the consequences of SERAT3;2 interaction with SERAT1;1 in a hetero-oligomeric SERAT complex *in vitro* and uncovered that SERAT3;2 inhibits the interaction of the hetero-oligomeric SERAT complex with OAS-TL A. Our data suggest that SERAT3;2 modulates the sensor function of the cytosolic CSC when sulfur supply is limited. The consequences of this modulatory impact on sulfur uptake and whole-plant sulfur distribution under sulfur-limiting conditions will be discussed.

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**REGULATION OF SULFATE METABOLISM BY O-ACETYLSERINE**

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The regulation of plant sulfate metabolism, especially under conditions when plants are exposed to sulfate deprivation, is still not entirely resolved and seems to be subject to a complex multi-facetted network. Sulfate deprivation induces a high number of genes, which actually reflects the involvement of sulfur in many diverse plant pathways. We focused our attention on a subgroup of these genes, which we termed OAS cluster genes. These genes are highly responsive to sulfate availability and are induced by O-acetylserine (OAS). OAS is the substrate for cysteine biosynthesis and accumulates under sulfate-deprived growth conditions, but seemingly also under other stress conditions. Among the OAS-cluster genes, we put special emphasis on understanding the function and regulation of the sulfur deficiency induced genes *SDI1* and *SDI2*. SDIs e.g. control the biosynthesis of glucosinolates through inhibiting MYB transcription factor activity (especially MYB28). In effect, this reduces flux into secondary sulfur pools in favour of primary metabolism when S is scarce. While glucosinolates are specific to *Brassicaceae,* SDIs are present in all plant genera we looked at. We identified a further function of SDIs as controlling the biosynthesis of sulfur rich seed storage proteins during seed development which might be a function shared by a wide range of plant species. Again, in concert with MYB transcription factors but also other seed metabolism regulators. Despite these findings, regulation by OAS is far from being completely understood and we will discuss in this session new findings and approaches.

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**FUNCTION AND REGULATION OF THE OAS CLUSTER GENES *SDI1* AND *SDI2*.**

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The regulation of plant sulfate metabolism, especially under conditions when plants are exposed to sulfate deprivation, is still not entirely resolved and seems to be subject to a complex multi-facetted network. Sulfate deprivation induces a high number of genes, which reflects the involvement of sulfur in many diverse plant pathways. We focus our attention on a subgroup of these genes, which we term OAS cluster genes. Why are they called OAS cluster genes and how were they defined?

OAS (O-acetylserine), the precursor of cysteine, is accumulating under –S conditions but not only. Conditions in which OAS content was elevated are light-dark shift, diurnal rhythm, *SERAT* overexpression lines. In experiments where the above three conditions were used while sulfur availability remains stable, OAS was accumulated in the plant tissue. Microarray analysis was conducted in those three experiments and six genes were identified whose expression was highly correlated with OAS accumulation. This set of genes was termed “OAS gene clus­ter” and includes genes encoding *APR3*, *SDI1*, *SDI2*, *SHM7*, ChaC and *LSU1*. These genes are not only strongly upregulated by sulfur deficiency, but their transcript levels also rise with endogenous increase of OAS at normal sulfate nutrition. Among the OAS-cluster genes, we put special emphasis on understanding the function of the sulfur deficiency induced genes *SDI1* and *SDI2* and embarked on understanding their regulation. *SDI1* transcripts can increase under –S conditions by more than 200-fold.

Analysis of *SDI1* mutants revealed that *SDIs* are involved in glucosinolate (GSL) biosynthesis and sulfur containing seed proteins. *SDI1* and *SDI2* are negative regulators of aliphatic and indolic GSLs accumulation under sulfur deficiency. The transcription factors (TFs) *MYB28, MYB29 and MYB76* were down-regulated in *SDI* overexpression lines. SDI1 forms a complex with MYB28, thus preventing its activation function of GSL biosynthesis genes. Additionally, it has been shown that *SDI1* downregulates another sulfur pool, the S-rich 2S albumin seed storage proteins in Arabidopsis seeds. In Ox*SDI1* lines, the protein content of 2S albumins was significantly reduced compared to *WT*. On the contrary, in Ox*MYB28* lines, the amount of 2S albumins was increased. Taken all these results into consideration, it was finally shown with Y3H that SDI1 downregulates 2S seed storage proteins by forming a ternary protein complex with MYB28 and MYC2.

In order to learn more about the regulation of the OAS cluster genes *SDI1* and *SDI2* we employed a Y1H screen using a transcription factor library, EMSA screening and a thorough *in silico* analysis of the promoter regions of both genes. We could identify 14 TFs binding to the respective promoters and scored their effect on the control of *SDI* gene expression. Especially interesting is that SLIM1 is among those TFs. We could show that additionally to the previously known *cis*-elements interacting with SLIM1/EIL3, UPE-box and TEBS, the long-known sulfur responsive element (SURE) interacts with SLIM1.

Currently we are expanding our research to i) understand better the function of SLIM1/EIL3 under different sulfur nutritional status and to ii) reveal additional functionalities of SDI1 under sulfur deprivation and external OAS application.

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TRANSCRIPTION FACTORS *SLIM1*, *RVE1* AND *RVE8* REGULATE OAS CLUSTER GENES

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The O-Acetylserine (OAS) cluster is a tightly co-regulated group of six genes whose expression is induced under sulfur deficiency, by OAS, and after transitioning from light to darkness (Hubberten et al., 2012). These genes are: S*ULFUR-DEFICIENCY-INDUCED 1 (SDI1)* and *2 (SDI2), ADENYLYL-SULFATE REDUCTASE 3 (APR3), LOW-SULFUR-INDUCED 1 (LSU1), SERINE HYDROXYMETHYLTRANSFERASE 7 (SHM7)* and *GAMMA-GLUTAMYL CYCLOTRANSFERASE 2;1 (GGCT2;1)*. To better understand how this cluster is regulated on a molecular level, we used the online tool Plant Regulomics (Ran et al., 2020) to find transcription factors binding to the corresponding promoters. Only seven factors were predicted to bind to all six promoters. Interestingly, one of them was *SULFUR LIMITATION1 (SLIM1)*, a key transcription factor in the regulation of sulfur deficiency response (Maruyama-Nakashita et al., 2006). We decided to also focus on *REVEILLE1 (RVE1)* and *REVEILLE8 (RVE8)*, since they are part of the circadian clock in *Arabidopsis thaliana*, a complex regulatory network which allows the plant to coordinate internal processes with their environment (Nohales et al., 2016). However, *RVE1* and *RVE8* are known for having additional functions (Rawat et al., 2009; Gray et al., 2017), therefore, we assessed them in the context of sulfur metabolism. Col-0, *slim1-1,* *rve1* and *rve8* *A. thaliana* seedlings were transferred from light to darkness, as well as fed with OAS to evaluate the induction of the OAS cluster genes. In addition, the response of *rve1* and *rve8* to sulfate deficiency was analysed. Our results show that the loss of function of *RVE1* and *RVE8* has a negative impact on sulfur metabolism, leading to reduced levels of sulfur containing metabolites as well as lower expression of various genes in the pathway. Nonetheless, the relative sulfur deficiency response is comparable to wildtype plants. On the other hand, the loss of function of *RVE1* or *RVE8* and the mutation of *SLIM1* prevented the transcriptional activation of the OAS cluster genes by light dark transition. We showed that *SLIM1* plays a key role in the translation of the OAS signalling, whereas *RVE1* and *RVE8* are not involved.Our work indicates a possible direct role of *RVE1* and *RVE8* in the regulation of sulfur metabolism, hinting at the connection of the pathway with the circadian clock, and also sheds some light on the mechanism of activation of the OAS cluster genes as well as the connection between *SLIM1* and OAS. Furthermore, our study lays the foundations for future experiments that will help to understand the complexity of the circadian clock and the link of its components and sulfur metabolism.

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**GENERATION OF SINGLE AND MULTIPLE ARABIDOPSIS *THALIANA* MUTANTS IN *LSU* (RESPONSE TO LOW SULFUR) GENES AND THEIR PRELIMINARY ANALYSIS**

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In Arabidopsis thaliana, there are four members of the plant-specific *LSU* (RESPONSE TO LOW SULFUR) gene family which are tandemly located on chromosomes 3 (*LSU1* and *LSU3*) and 5 (*LSU2* and *LSU4*). The LSU proteins consist of about 100 residues and they contain the predicted coiled-coil structures. In accordance, they are able to form homo- and heterodimers. The molecular functions and importance of LSU proteins are still unknown however there is some evidence that they are involved in plant responses to environmental challenges, such as sulfur deficiency and plant immunity. A set of lsu deletion lines, including the single, double, triple and a quadruple mutants were obtained using the CRISPR/Cas9 technology. The deletion regions in the obtained lines were mapped and the level of expression of each *LSU* transcript was assayed in each line. Preliminary tests of seedlings growth in nutrient deficient media failed to detect any substantial differences of the lsu mutants from the wild type. The further analysis of the lines is in progress. The main focus is on the quadruple mutant, including its detailed phenotypic and morphological analysis, as well as monitoring differences in gene expression in sulfur sufficient and deficient medium in comparison to the wild-type plants.

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**ANALYSIS OF THE PROMOTER REGIONS OF *LSU* (*RESPONSE TO LOW SULFUR*) GENES IN *Arabidopsis thaliana***

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*LSU* genes encode small coiled coil proteins and at least two of them, *LSU1* (At3g49580) and *LSU2* (At5g24660), were initially found to be up-regulated in low sulfur conditions. The information about the expression of the other two, *LSU3* (At3g49570) and *LSU4* (At5g24655), was not available at that time. Now, more conditions affecting expression of *LSU* genes have been identified. Among others, induced transcript levels of *LSU* genes were found also in such conditions as oxidative stress or the beginning of a darkness period. Interestingly, *LSU1* was identified as a member of the set of OAS cluster genes whose levels of expression corresponded to the level of O-acetylserine (OAS). The level of OAS increases upon sulfur starvation and shortly after transition to the darkness. It is an important metabolite of sulfur assimilation and the direct precursor of cysteine. Nevertheless, the significance of this possible regulation remains elusive and its molecular details are unknown. In this work we focused on the promoters of *LSU* genes. The promoter regions of *LSU1*, *LSU2*, *LSU3* and *LSU4* have been fused to reporter genes encoding GFP and GUS, and the expression cassettes were introduced into *Arabidopsis* plants. Then, the activity of the promoters was monitored in seedlings maintained in different growth conditions. The results of this analysis was compared with expression of *LSU* genes from numerous publicly available microarrays and RNAseq data sets. The aim of this analysis was identification of the main *cis*- and *trans-*factors responsible for regulation of expression of LSU genes*.* Binding of the selected factors to the promoter regions of *LSU* genes was examined by yeast-one-hybrid method and *in vitro* by electrophoretic mobility shift assay (EMSA). The putative binding motifs in the promoter of *LSU1* were verified by targeted mutagenesis.

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**SELECTIVE AUTOPHAGY CARGO RECEPTOR MODULATES SULPHUR STARVATION RESPONSE IN *Arabidopsis thaliana***

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Autophagy is implicated in almost every aspect of plant growth, from embryogenesis to senescence and in numerous stress responses. Arabidopsis mutants defective in the autophagy system (e.g. atg5) are hypersensitive to carbon and nitrogen starvation, and display early senescence even under nutrient-rich conditions. A variety of stressful conditions, such as nitrogen or carbon starvation, oxidative stress, ER stress, heat, drought, saline stress, osmotic stress, sugar excess and senescence induce autophagic flux.

Recently, links between autophagy and plant response to sulphur deficit have been demonstrated by Dong et al. 2017 [1]. These authors noticed that sulphur starvation blocks the activity of TOR kinase via not well-characterized downregulation of glucose metabolism. The downregulated TOR activity caused, as expected, reduced translation, lowered meristematic activity and elevated autophagy.

On the other hand, we have observed that plants exposed to sulphur deficit elevate the transcription of *NBR1* gene encoding selective autophagy cargo receptor which might reflect an increased demand for NBR1 in such conditions. Therefore, we investigated the role of this selective autophagy cargo receptor in plant response to sulfur deficit (dS). Transcriptome analysis of the wild-type and NBR1 overexpressing plants pointed out differences in gene expression in response to dS. Our attention focused particularly on the genes upregulated by dS in the roots of both lines because of the significant overrepresentation of cytoplasmic ribosomal gene family. Moreover, we noticed an overrepresentation of the same family in the set of proteins co-purifying with NBR1 in dS. One of these ribosomal proteins, RPS6 was chosen for verification of its direct interaction with NBR1 and proven to bind outside the NBR1 ubiquitin-binding domains. The biological significance of this novel interaction and the postulated role of NBR1 in ribosomes remodelling in response to starvation remain to be further investigated. Interestingly, NBR1 overexpressing seedlings have significantly shorter roots than wild-type when grown in nutrient-deficient conditions in the presence of TOR kinase inhibitors. This phenotype probably results from excessive autophagy induction by the additive effect of NBR1 overexpression, starvation, and TOR inhibition [2].

Previously, we reported that expression of the gene encoding the selective autophagy cargo receptor Joka2 (NtNBR1) in tobacco was induced in plants exposed to sulphur deficit [3]. The links between plant NBR1 and sulphur availability were additionally suggested by the fact that Joka2 was identified as a partner of the UP9C protein encoded by a gene strongly induced by sulphur starvation. The UP9C protein is a member of the plant-specific family of LSU (Response to Low SUlfur) –like proteins, identified as important stress hubs involved in multiple protein-protein interactions [4, 5, 6]. Interestingly, *LSU1* belongs to the OAS cluster genes in Arabidopsis [7].

Additionally, our recent observation that the NBR1 autophagy cargo receptor is responsible for cross-talk between autophagy and the ABA signalling pathway adds this hormone as an additional player in sulphur deficiency response. Overall, the excess or the lack of NBR1 protein in Arabidopsis enhances or reduces ABA signalling, respectively [8].

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**MANIPULATING SULFUR METABOLISM TO ENHANCE HEAVY METAL TOLERANCE AND IMPROVE SAFETY AND NUTRITION OF RICE**

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Heavy metals and metalloids, such as cadmium (Cd) and arsenic (As), are highly toxic to living organisms. Widespread As contamination of rice paddies has been documented in south and southeast Asia, causing phytotoxicity and substantial yield losses. In addition, both As and Cd are readily taken up by rice plants and accumulated in the grain, posing considerable risks to human health. Rice, the staple food for over half of the global population, is a major dietary source of both As and Cd. On the other hand, around 75% of the rice grains produced globally have insufficient levels of selenium (Se) to meet human’s requirement. It is therefore important to understand how rice plants take up, transport, and detoxify As and Cd. We used a forward genetic approach by isolating As and Cd tolerant mutants of rice and found that both are related to altered sulfur (S) metabolism. The As tolerant mutant (*astol1*) showed a dominant inheritance, suggesting a gain-of-function in As tolerance. The mutant also showed decreased As accumulation, but increased S and Se accumulation in rice grain. *OsASTOL1* encodes a chloroplast-localized *O*-acetylserine (thiol) lyase (OAS-TL) and the mutant has a point mutation resulting in a substitution of Ser189Asn. Although the mutant protein lost the OAS-TL activity, it interacts more strongly with its partner serine-acetyltransferase (SAT) in the cysteine synthase complex, resulting in enhanced SAT activity and consequently increased synthesis of OAS, Cys, glutathione and phytochelatins, as well as increased uptake of sulfate and selenate. Increased levels of phytochelatins not only lead to enhanced tolerance to As, but also decreased As translocation to the grain. In the Cd tolerant recessive mutant (*cadt1*), the casual gene encodes a putative serine hydroxymethyltransferase, which is localized to the nucleus. Mutation in *OsCADT1* also resulted in higher S and Se accumulation in rice shoots and grains, due to increased expression of the sulfate transporter gene *OsSULTR1;1* and the S-deficiency inducible gene *OsSDI1*. Thiol compounds including cysteine, glutathione and phytochelatins were significantly increased in the mutant, underlying its increased Cd tolerance. OsCADT1 appears to act as a negative regulator of sulfate uptake and assimilation, although the exact mechanism remains unclear. Results from both studies indicate that the S uptake and metabolism pathway can be manipulated to increase the detoxification capacity against As and Cd, with the additional benefit of decreased accumulation of As but increased accumulation of Se in rice grain.

**HIGH LEVELS OF METHIONINE IN ARABIDOPSIS SEEDS RESULT IN HIGHER INFLUX OF NUTRIENTS FROM THE LEAVES TOWARD THE SEEDS**

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Studies have shown that expression of the key gene of methionine synthesis, cystathionine gamma synthase (CGS), under the control of the phaseolin promoter in Arabidopsis plants (SSE plants) causes a significant increase of about 6-fold in methionine levels in the seeds. This increase is accompanied by an increase in other amino acids (AAs) in the seeds and a marked increase in sugar levels (Cohen et al. 2014). Specific metabolites whose levels have increased in seeds also increase when plants are exposed to abiotic stress. Indeed, in SSE dry seeds, the expression levels of genes related to ABA and ethylene, which are both related to stresses, are significantly increased relative to seeds containing empty vector (EV) (Cohen et al. 2014). The increase in the levels of AAs and sugars in SSE seeds was not accompanied by the increase of biosynthetic genes associated with the formation of these metabolites. This suggests that these metabolites might import from the vegetative tissues. If that indeed occurs, SSE leaves and siliques should have more metabolites than EV. To test this assumption, the SSE and EV plants were tested at three developmental stages when the seeds are formed. It was found that SSE leaves and siliques contain significantly higher levels of AAs and sugars relative to EV at the three stages of development. This finding indicates an increased flux of metabolites from the vegetative tissues towards the developing seeds of SSE. To test it further, a feeding experiment was performed in which isotope-labeled aspartate and glutamate were inserted into the leaves and siliques. After ten hours, the labeled AAs were measured in the seeds. The results showed that SSE seeds have significantly higher contents of the two labelings AAs than EV seeds, suggesting that the flux towards seeds of SSE is higher than EV. RNA SEQ analyses were done on the leaves of SSE to understand better the rise of metabolites in the leaves of SSE. The analysis is performed at the last stage of plant development when the leaves begin to senescence, and most of the metabolites are transferred towards the seeds. The analysis showed that SSE leaves have more decomposition of polymers along with a continued synthesis of monomers compared to EV. In addition, many genes related to stresses and ABA have risen in SSE leaves, and there is more expression of genes encoding transporters. This finding is consistent with the metabolic results detected in leaves and seeds of SSE. The changes in SSE leaves are not expected since the phaseolin promoter is known to express only in seeds (Fait et al. 2011). To understand why the leaves express genes associated with synthesis of metabolites, it was decided to examine the leaves of SSE plants in the early, pre-flowering stages. The results showed a high level of methionine, other AAs, and a high expression level of CGS. The results indicate that the phaseolin promoter is active in the leaves of SSE plants. Previously it was shown that this promoter is not active in Arabidopsis leaves since it is stringently turned off during all vegetative stages of plant development by the presence of a nucleosome that is positioned over its TATA regions (Li et al., 1998). However, when the level of ABA increased and other ABA-related proteins accumulated, this promoter was found to be active in Arabidopsis leaves (Sundaram et al. 2013). Examination of the methylation level of the promoter showed that it is more methylated in SSE relative to EV. Since nucleosomes protect DNA from DNA methylation (Felle et al. 2011), and the phaseolin promoter was methylated, we assume that this promoter is active in the leaves of SSE plants, causing a high level of methionine. A high level of methionine probably affects the formation of metabolites associated with stresses. These metabolites are then channelled from the leaves towards developing seeds that act as strong sinks at this developmental stage.

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**γ-GLUTAMYL PEPTIDASE 1 DEGRADES GLUTATHIONE AND PARTICIPATES IN BOTH PRIMARY AND SECONDARY SULFUR METABOLISM.**

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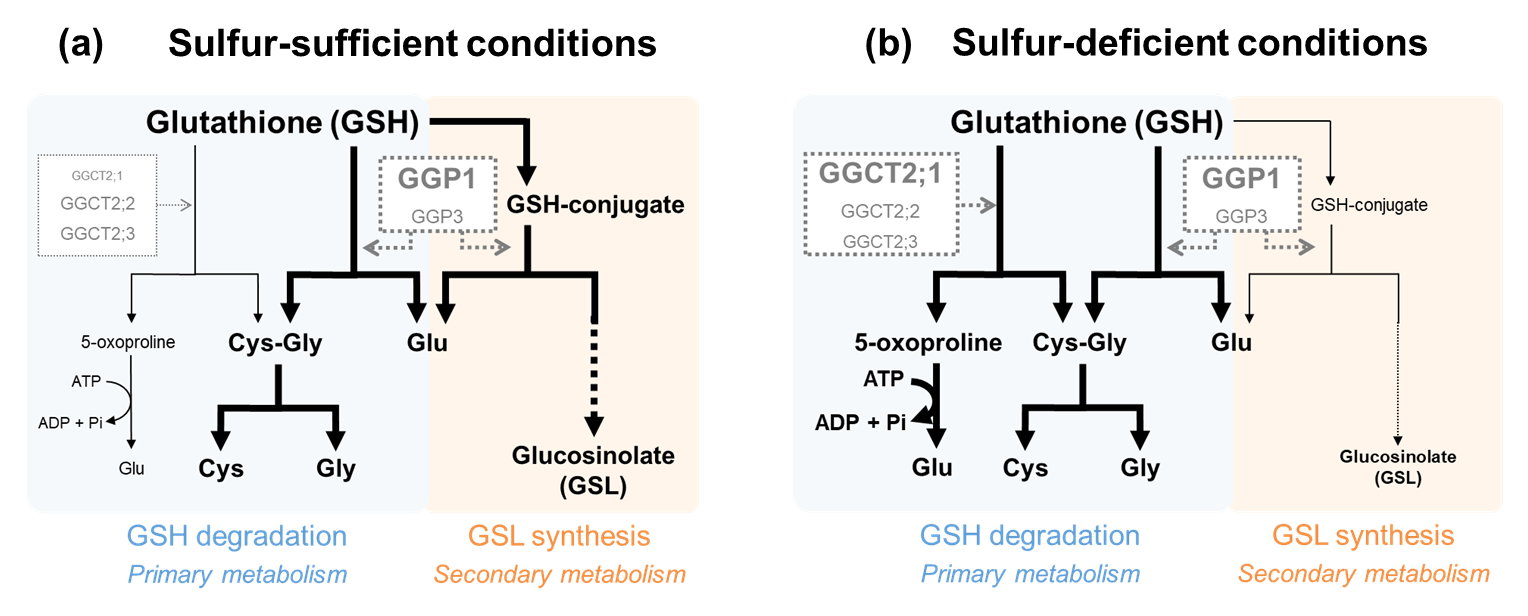
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Glutathione (GSH; γ-glutamyl-cysteinyl-glycine) is a well-known redox buffer in the cell, but it also plays an important role in primary sulfur metabolism. GSH contains Cys residue and functions as a major Cys repository. It can therefore be said that correct understanding of GSH metabolism is required to improve plant stress tolerance and sulfur utilization efficiency to achieve sustainable agriculture. In this study, we introduce a newly discovered GSH degradation activity of γ-Glutamyl Peptidases(GGPs), enzymes involved in glucosinolate (GSL) synthesis, and present a hypothetic model of GSH degradation and GSL synthesis in *Arabidopsis* (Figure 1). The model contains two major claims: (i) GGP1 is a functional, and possibly primary, GSH degradation enzyme. (ii) GGP1, and possibly GGP3, plays a role in both GSH degradation and GSL and camalexin synthesis pathways. What is interesting about the first point is that GGPs could render a more efficient route for GSH degradation than already-known GSH degradation enzymes, γ-glutamyl cyclotransferases (GGCTs). GGP pathway does not require ATP whereas GGCT pathway does, so it seems reasonable if GSH is basically degraded through more efficient GGP pathway. Also, the second point indicates that GGPs play dual functions in primary and secondary sulfur metabolisms. It seems even possible that GGPs enable plants to coordinate these sulfur metabolisms in response to environmental conditions.

In this study, we firstly prepared yeast mutants defective in GSH utilization and transformed *Arabidopsis* cDNA library into the mutants. The result showed that the phenotype was complemented in all transformants by the same gene, *γ-Glutamyl Peptidase 1*(*GGP1*), even though GSH degradation had been believed to be carried out by GGCTs. To examine whether GGP1 could really be functional in the cell, GSH degradation activity of GGP1 was analyzed using recombinant proteins, together with its homolog GGP3. Calculated *Km* and *kcat* values were in favor of GSH degradation by GGPs considering cytosolic GSH concentration. In search of direct evidence of GSH degradation by GGPs, GSH concentration in the *ggp1* null mutants was investigated. As a result, these mutants showed higher GSH concentration than the wild-type plant under control, sulfur-deficient, and nitrogen-deficient conditions. Characteristics of GGPs and GGCTs were further investigated in terms of expression. The *GGP1* transcripts were highly abundant, especially in rosette leaves, which was in agreement with the result of yeast complementation assay or constant GSH accumulation in the *ggp1* mutants. Additionally, we conducted metabolome analysis to seek further proofs of GSH degradation by GGP1 and to understand broad impacts of GSH degradation. There were several points worth noting, but it was of particular importance that the *ggp1* mutant accumulated more *O*-acetylserine (OAS) than the wild-type plant under sulfur deficiency. OAS is a signaling molecule for sulfur starvation, so this result implies that the GGP1 disruption caused severer sulfur-deficiency stress. Finally, because GGPs also process GSH-conjugates in GSL and camalexin synthesis pathways, we examined the feasibility of their dual functions in GSH degradation and GSL and camalexin synthesis by predicting three-dimensional structure of GGPs. The result indicated that GGPs seem to be able to recognize both GSH and GSH-conjugates, supporting our hypothesis of dual functions.



**Figure 1.** Models of GSH degradation and GSL synthesis in *Arabidopsis*. (a) Under sulfur-sufficient conditions, GGP1 is estimated to play a central role in GSH degradation and to process GSH-conjugates in the GSL synthesis pathway. (b) Under sulfur-deficient conditions, the expression of *GGCT2;1* is promoted, and GGCT2;1 accelerates GSH degradation. GSL synthesis is reduced under these conditions.

Acknowledgements & Funding

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*Ito, T., Kitaiwa, T., Nishizono, K., et al. (2021) Characterization of γ-Glutamyl Peptidases and γ-Glutamyl Cyclotransferases for Glutathione Degradation in Arabidopsis. bioRxiv, 2021.10.29.466391.*

**PHYSIOLOGICAL ANALYSIS OF THE CYSTEINYLGLYCINE DEGRADING ENZYME THAT FUNCTION IN GLUTATHIONE DEGRADATION IN *Arabidopsis thaliana***

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In plants, sulfur (S) absorbed as sulfate ions is assimilated into cysteine (Cys), and Cys is incorporated into glutathione (GSH) as a storage form of S, which is transported to the necessary tissues depending on nutritional conditions. The transported GSH is degraded to supply Cys to the tissues for synthesizing proteins and various S-containing compounds. Thus, degradation of GSH has an important role in S metabolisms in plants. Degradation of GSH to cysteinylglycine (CysGly) is performed by ɤ-Glutamyl peptidase or ɤ-Glutamyl cyclotransferase in plants and their contributions depend on organs or environmental conditions (Itoh et al., 2021). In the present study, we focused on the next step, degradation of CysGly.

In *Saccharomyces cerevisiae*, GSH is degraded by the complexed proteins of DUG2-DUG3 (DUG: Defective in Utilization of Glutathione) and CysGly is then degraded by DUG1 (Ganguli et al., 2007). In the screening of *Arabidopsis thaliana* cDNA library, the *AtDUG1* gene complemented yeast *dug1Δ* mutant which is defective in Cys-Gly degradation and enable to grow on the medium with GSH as a sole S source. AtDUG1 is predicted to localize in the cytosol.

To know the contribution of AtDUG1 for CysGly degradation *in planta*, the activity assay of CysGly degradation was compared between wild-type and *atdug1* knockout and RNAi mutant plants. The *atlap1* knockout plants were also included as the *AtLAP1* gene was previously reported to complement yeast *dug1Δ* mutant and its recombinant protein carry CysGly degradation activity (Kumar et al.,2015). In the protein extracts from 3-week-old plant leaves, CysGly degradation activity was significantly decreased in the *atdug1* knockout and the RNAi mutant plants about 30 % compared to the wild-type plants. While there was no significant decrease of CysGly degradation activity in the *atlap1* mutant plants compared to wild-type plants. This indicates that AtDUG1 protein contribute CysGly degradation *in planta*.

Next, CysGly degradation activities were compared between wild-type and *atdug1* knockout in various organs. In 40-day-old plants. CysGly degradation activities of *atdug1* knockout were significantly decreased compared to that of wild-type plants in flowers, siliques and mature leaves while no difference was observed in stems and young leaves. These trends were corresponded to the expression patterns of the *AtLAP1* gene in the eFP Browser (Klepicova et al. 2016). As this gene is also reported to express highly in seeds in the eFP Browser, CysGly degradation activities were compared in seeds before and during germination. CysGly degradation activities of *atdug1* knockout were significantly decreased compared to that of wild-type plants in germinated seeds at 1, 2 and 3 days after imbibition. This implies that AtDUG1 have roles to provide Cys by degrading CysGly for synthesis of S-containing compounds during germination. Significant increase of CysGly and degrease of Cys concentrations were observed in germinated seeds of *atdug1* knockout plants compared to wid-type plants, supporting the hypothesis. Furthermore, length of main roots of *atdug1* knockout were significantly longer than those of wild-type plants at 2 and 3 days after germination. The elongation of main roots is considered to be a response to S-deficiency as shown in Zhao et al., 2014.

To know the contribution of AtDUG1 in plant metabolites, widely targeted metabolome analysis (Sawada et al. 2009) was performed using wild-type and *atdug1* knockout plants under Control, S-deficient and N-deficient conditions. Under S-deficient condition, *O*-acetyl-L-Ser (OAS), a signal metabolite for S-deficiency, was significantly increased in *atdug1* knockout plants than in wild-type plants, indicating that defects in providing Cys from CysGly in *atdug1* knockout plants resulted in more severe S-deficiency.

From these results, it was considered that AtDUG1 contributes to provide Cys from CysGly in GSH degradation pathway which is especially important in germination.

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**DISRUPTION OF SULFUR DEFICIENCY-INDUCED GLUCOSINOLATES CATABOLISM affects glucosnilates distribution in mature plants**

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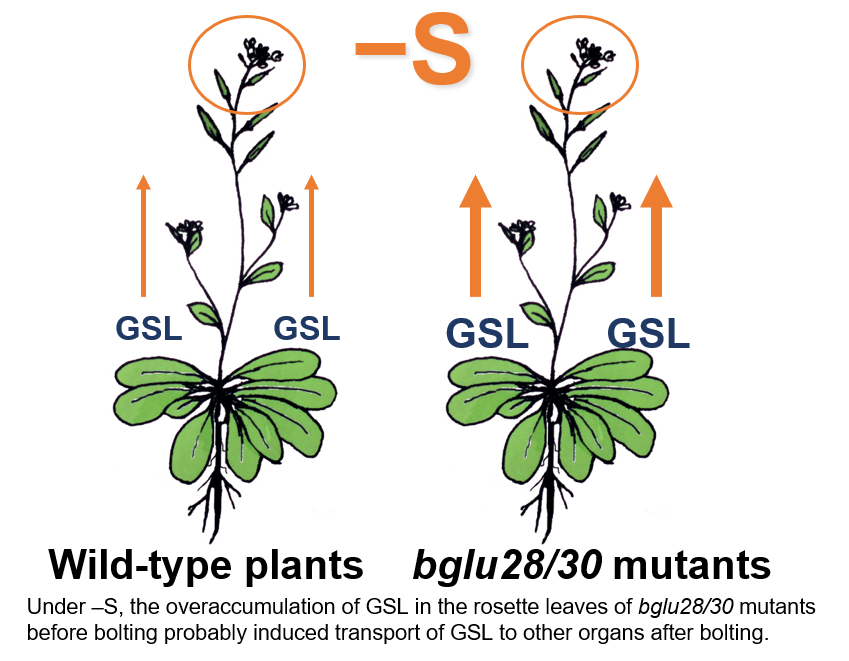
In agriculture, S deficiency (−S) can greatly impair crop yield and quality. To survive under −S, plants will prioritize allocating S to primary S metabolism, by which the compounds essential for plant growth, such as cysteine and glutathione, are synthesized (Maruyama-Nakashita 2017). Conversely, the biosynthesis of secondary S-containing compounds, glucosinolates (GSL), the specialized metabolites in Brassicaceae species, is repressed. Meanwhile, their catabolism is stimulated. As a result, GSL level was drastically reduced under −S. However, the regulatory mechanisms and physiological significance of altered GSL metabolism under −S is not well-explained yet, especially about the −S-induced GSL catabolism.

GSL catabolism requires a group of ß-glucosidases (BGLU) named myrosinases. The upregulation of the transcript levels of two putative myrosinases, *BGLU28* and *BGLU30*, implied their potential roles in GSL catabolism under −S (Maruyama-Nakashita 2017). Thus, previously we explored the −S-induced GSL catabolism by characterizing the functions of BGLU28 and BGLU30. We generated the *bglu28/30* double knockout mutants and found that, in *bglu28/30* seedlings, GSL level was significantly increased, while some primary S-containing compound levels were decreased under −S. Furthermore, *bglu28/30* displayed apparent growth retardation under −S. These results indicated that BGLU28 and BGLU30 are responsible for GSL catabolism under −S, by which S released from GSL is recycled to synthesize primary S-containing compounds that are essential for plant growth (Zhang et al. 2020, Sugiyama et al. 2021).

Based on the above findings, as GSL accumulation is distinct among different plant organs and growth stages (Brown et al. 2003), we further investigated the physiological roles of BGLU28 and BGLU30 in mature plants grown under different S conditions. Under −S, mature *bglu28/30* mutants also showed impaired growth. The fresh weight of their rosette leaves, dry weight of aerial part and seed yield was reduced. To better characterize the function of these two BGLUs, we then observed their tissue-specific expressions. We found that both *BGLU28* and *BGLU30* are expressed in rosette leaves. In other tissues, *BGLU28* is expressed in cauline leave, siliques and flowers, while *BGLU30* is specifically expressed in the young cauline leaves.

Consistent with the expression pattern of *BGLU28* and *BGLU30*, GSL level was also increased in those plant tissues of the *bglu28//30* mutants grown under −S. However, noticeably, the increase of GSL in rosette leaves was only observed before bolting, but not after bolting. Since GSL can be transported from rosette leaves to other aerial parts after bolting, we suppose such a transport process might be stimulated in *bglu28/30* mutants under −S. To test this hypothesis, we supplied allyl GSL (not produced in Arabidopsis Columbia) on the rosette leaves of plants. After 72 hours of treatment, we found more allyl GSL was translocated in the siliques and flowers of *bglu28/30* mutants under −S compared to that in wild-type (WT) plants.

These results indicated that BGLU28/30 functions in plants at both vegetative and reproductive stages to support plant growth under −S. The distinct expression pattern of *BGLU28/30* in the mature plants suggested that they work separately but coordinately to modify GSL profile. Moreover, the disruption of the *BGLU28/30* resulted in an enhanced GSL transport process under −S, hinting that more unexpected metabolic changes might happen in these *bgu28/30* mutants. Overall, our results revealed the physiological functions of BGLU28 and BGLU30 and deepened our understanding of plant adaptation strategies to S stress.



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**Acknowledgements**

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**CYSTEINE DESULFHYDRASE LCD1 INTERACTS WITH IMPORTIN α3 AND REGULATES TOMATO FRUIT RIPENING AND SENESCENCE IN TOMATO**

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In recent years, accumulating studies have found that hydrogen sulfide (H2S) could delay the ripening and senescence of various fruits and vegetables, and the cysteine desulfhydrase LCD1 is a key enzyme in the production of endogenous H2S in plants (Li et al., 2014; Yao et al., 2018). Previously, we found that LCD1 in tomato is localized in the nucleus, and LCD1 deletion significantly accelerated the ripening and senescence of tomato (Hu et al., 2020).

Here we found the interaction between the nuclear import protein importin α3 and LCD1 by using yeast two-hybrid. Subsequently, the luciferase complementation assay was used to verify the interaction between importin α3 and LCD1. Using virus-induced gene silencing (VIGS), the mechanism of importin α3 in regulating fruit ripening was studied. The study found that importin α3 silencing accelerated fruit ripening, accelerated chlorophyll degradation, and increased the expression levels of fruit ripening marker genes chlorophyll degradation genes (*PPH*, *SGR1*, *NYC1*, *PAO*), carotenoids and genes (*ZDS*, *PSY*, *PDS*) and ethylene pathway genes (*ACS2*, *ACO3*, *ACO1*, *RIN*, *E8*, *E4*, *NOR*). Using Crispr/Cas9 technology, tomato *importin α3* gene-edited plants were constructed, and it was found that importin α3 deletion led to premature ripening of tomato fruit. Using *importin α3* mutant and wild-type tomato plants as experimental materials, LCD1-GFP was transiently expressed, and it was found that importin α3 deletion caused part of LCD1-GFP to migrate from nucleus into the cytoplasm, further indicating the regulatory effect of importin α3 on the nuclear localization of LCD1-GFP.

In conclusion, through the construction of importin α3 transiently silenced tomato fruit, importin α3gene-edited plants, and the determination of physiological and biochemical index related to ripening and senescence, it is clear that both LCD1 and importin α3 play an important regulatory role in the process of tomato ripening and senescence.

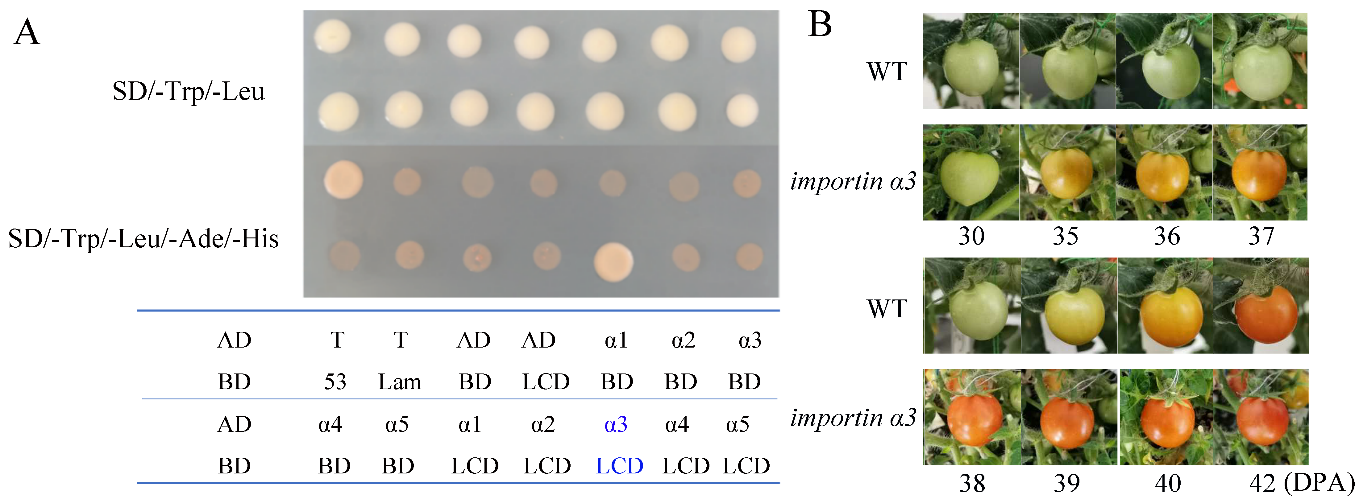


Figure 1. (A) By yeast two-hybrid, the interaction between importin α3 and LCD1 is confirmed. (B) *importin α3* deletion mutant constructed by Cripsr/Cas9 shows accelerated fruit ripening as showed by images from 30 to 42 days post anthesis.

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ANTHOCYANIN PRODUCTION IN *Arabidopsis* ROOTS USING THE DOWNSTREAM REGION OF SULFATE TRANSPORTER *SULTR2;1*

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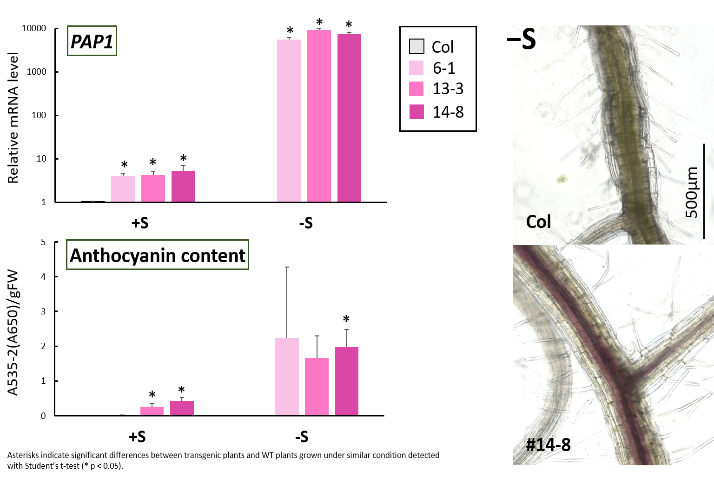
High-level expression system in plants is essential for basic research or to enhance a desired trait. However, most systems require modification of the gene’s upstream region, which can result in loss of expression characteristics, unfavorable background expression due to non-native promoter or promoter homology-based silencing in case of gene stacking.

SULTR2;1 is a low-affinity sulfate transporter of *Arabidopsis thaliana*. Under sulfur deficiency (−S), *SULTR2;1* expression is highly up-regulated in roots due to the 3’downstream region (*TSULTR2;1*). *TSULTR2;1* can also increase other genes’ expression regardless of their promoter sequences in roots under −S. Based on these unique characteristics, we were motivated to apply this sequence for establishing a regulatory gene expression system by sulfur concentration. Hereby, gene’s upstream region can be conserved, and the induced expression will be root specific.

To test the system, we chose *PAP1*, a positive regulator of anthocyanin biosynthesis. Anthocyanin is a flavonoid that induces the red, purple, or blue pigments in higher plants. It is also a valuable antioxidant for both plants and human consumption.

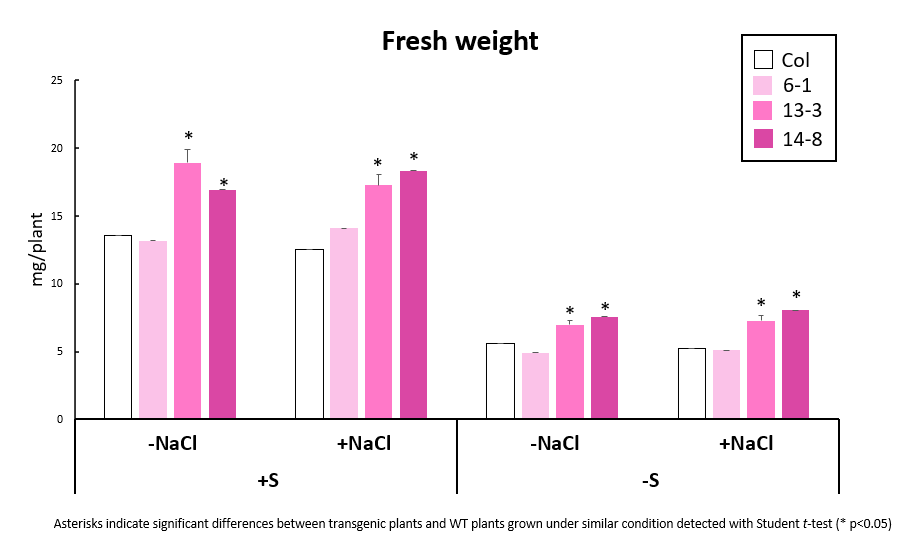
5’upstream and coding region of *PAP1* were fused to *TSULTR2;1*, and the construct was introduced into *Arabidopsis thaliana* ecotype Columbia. Three lines with highest *PAP1* expression level, #6-1, #13-3, and #14-8, were selected and sown on MGRL agar media supplemented with 1500 µM (+S) or 15 µM sulfate (−S) with the wild-type (WT). Under −S, the transgenic lines dramatically induced *PAP1* mRNA level in the roots and anthocyanin content to 2-3 times higher than that in WT (Figure 1). Roots of the transgenic plants under −S developed a pink-purplish color due to anthocyanin accumulation, while the roots of WT remained white (Figure 1).

**Figure 1**. −S-induced *PAP1* expression, anthocyanin accumulation and the root color.



We also tested whether *PAP1* expression could be transiently up-regulated by shifting the plants from +S to −S conditions. As a result, the root color became pink four days after the shift. Both *PAP1* expression and the anthocyanin level were increased with the increased expression of *PAP1*-regulated anthocyanin biosynthetic genes (*DFR, ANS*) in the roots.

As anthocyanin is known to scavenge the reactive oxygen species accumulated in the vacuoles, thus, improves plants’ tolerance to abiotic stress. The salt stress tolerance of *PAP1* transgenics was assessed by growing plants on +S and −S media supplemented with 50 mM NaCl. Transgenic lines showed better growth in the stress environment than WT plants, correlating with higher root antioxidant activity, suggesting that anthocyanin accumulation could improve plants’ stress tolerance.



**Figure 2.** Transgenic plants express higher tolerance to salt stress

The results of this study indicated the usefulness of *TSULTR2,1* for tentative gene expression technology. The method is promising to be applied to improve the nutrient quality, stress-tolerant ability, or change the color of commercial root vegetable crops.

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**SULFATE-TOR SIGNALLING CONTROLS TRANSCRIPTIONAL REPROGRAMMING FOR SHOOT APEX ACTIVATION**

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Plants play a primary role for the global sulfur cycle in the earth ecosystems by reduction of inorganic sulfate from the soil to organic sulfur-containing compounds. How plants sense and transduce the sulfate availability to mediate their growth remains largely unclear. The target of rapamycin (TOR) kinase is an evolutionarily conserved master regulator of nutrient sensing and metabolic signalling to control cell proliferation and growth in all eukaryotes. Here, we report that inorganic sulfate exhibits high potency activating TOR and cell proliferation in the leaf primordium to promote true leaf development in *Arabidopsis* in a glucose-energy independent pathway. Genetic and metabolite analyses further suggested that this sulfate activation of TOR is independent from the sulfate-assimilation process and glucose-energy signalling. Significantly, tissue specific transcriptome analyses uncover previously unknown sulfate-orchestrating genes involved in DNA replication, cell proliferation and various secondary metabolism pathways, which largely depends on TOR signalling. Systematic comparison between the sulfate- and glucose-TOR controlled transcriptome further reveals that TOR kinase, as the central growth integrator, responds to different nutrient signals to control both shared and unique transcriptome networks, therefore, precisely modulates plant proliferation, growth and stress responses.

LOCAL AND SYSTEMIC RESPONSE TO HETEROGENEOUS SULFATE RESUPPLY AFTER SULFUR DEFICIENCY IN RICE

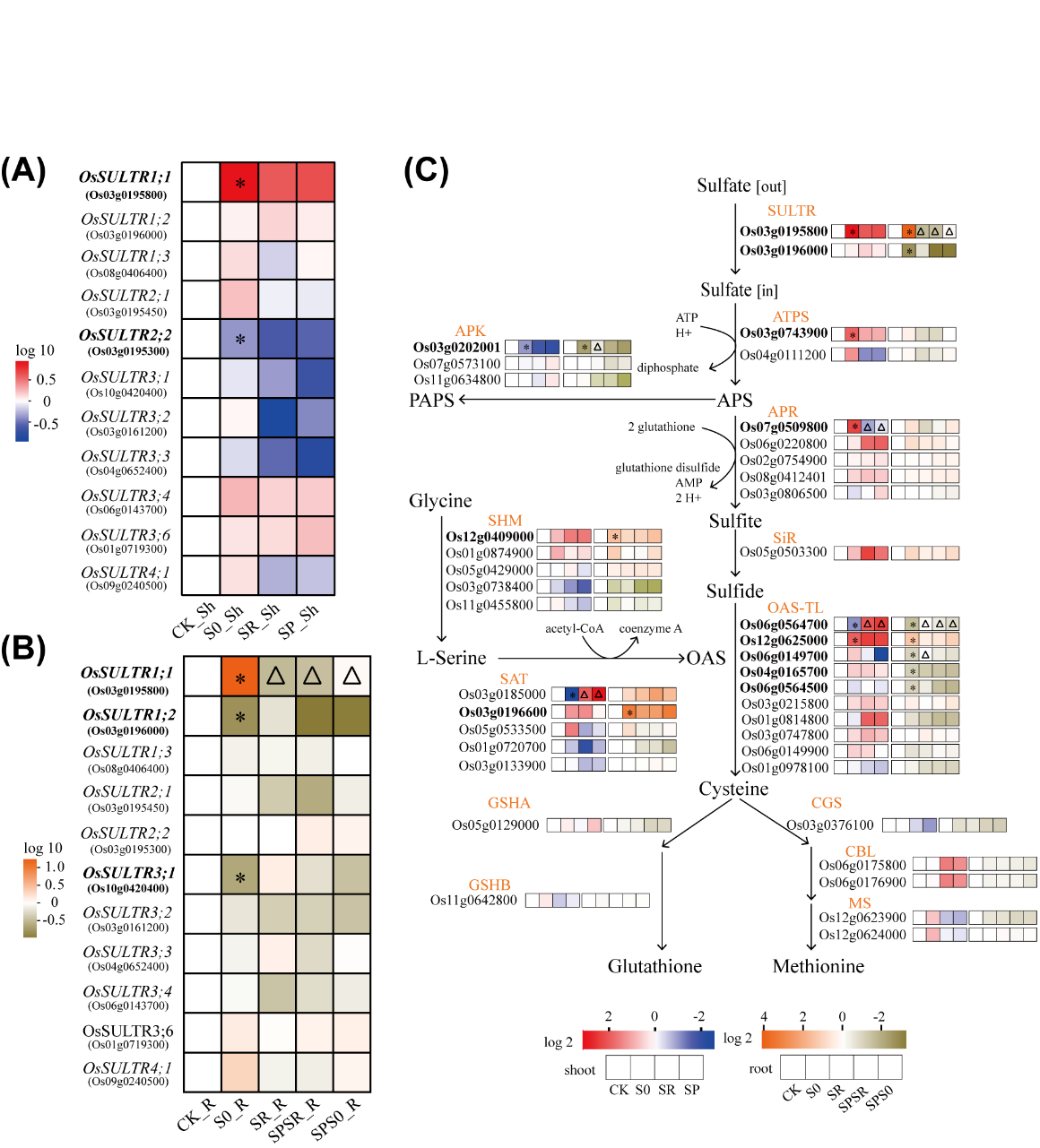
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Sulfur (S) is an essential mineral nutrient required for plant growth and development. Plants usually face temporal and spatial variation in sulfur availability, including the heterogeneous sulfate content in soils. As sessile organisms, plants have evolved sophisticated mechanisms to modify their gene expression and physiological processes in order to optimize S acquisition and usage (Takahashi et al., 2010). Such plasticity relies on a complicated network to locally sense S availability and systemically response to S status, which remains poorly understood. Here, we took advantage of a split-root system and performed transcriptome-wide gene expression analysis on rice plants in S deficiency followed by sulfate resupply. S deficiency altered the expressions of 6749 and 1589 genes in roots and shoots, respectively, accounting for 4.28% and 18.07% of total transcripts detected. Homogeneous sulfate resupply in both split-root halves recovered the expression of 27.06% of S deficiency responsive genes in shoots; while 20.76% of S deficiency responsive genes were recovered by heterogeneous sulfate resupply with only one split-root half being resupplied with sulfate. The local sulfate resupply response genes with expressions only recovered in the split-root half resupplied with sulfate but not in the other half remained in S deficiency were identified in roots, which were mainly enriched in cellular amino acid metabolic process and root growth and development. Several systemic response genes were also identified in roots, whose expressions remained unchanged in the split-root half resupplied with sulfate but were recovered in the other split-root half without sulfate resupply. The systemic response genes were mainly related to calcium signaling and auxin and ABA signaling. In addition, a large number of S deficiency responsive genes exhibited simultaneous local and systemic response to sulfate resupply, such as the sulfate transporter gene OsSULTR1;1 and the O-acetylserine (thiol) lyase gene (Figure 1), highlighting the existence of systemic regulation of sulfate uptake and assimilation in S deficiency plants followed by sulfate resupply. Furthermore, knockout of the sulfate transporter family I members individually (*ossultr1;1*, *ossultr1;2* or *ossultr1;3* single mutants) or simultaneously (*ossultr1;1 1;2* double mutant or *ossultr1;1 1;2 1;3* triple mutant) disrupts the expressions of some local and/or systemic response genes under S deficiency and sulfate resupply, suggesting the involvement of sulfate transporter family I proteins in mediating the response to S deficiency. Our studies provided a comprehensive transcriptome-wide picture of local and systemic response to heterogeneous sulfate resupply which will facilitate the understanding of systemic regulation of S homeostasis in rice.

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**Figure 1. Summary of expression pattern of genes involved in sulfate uptake, assimilation, and metabolism in roots and shoots of plants under various S conditions.** (**A**) Relative expression level of sulfate transporter genes in shoots of rice plants under S deficiency or resupplied with sulfate homogeneously or heterogeneously in roots. (**B**) Relative expression level of sulfate transporter genes in roots of rice plants under different S conditions. (**C**) Relative expression level of genes involved in sulfate assimilation and metabolism in roots (right panel) and shoots (left panel) of plants under different S conditions. Relative expression level in (**A**-**C**) were normalized to the control (CK). Asterisks (\*) represented genes that were significantly differentially expressed under S deficiency. Triangles (∆) represented genes showing local, systemic or both response to sulfate resupply. SULTR: sulfate transporter; ATPS: ATP sulfurylase; APR: adenosine-5‘-phosphosulfate reductase; APK: adenosine-5’-phosphosulfate (APS) kinase; SIR: sulfite reductase; OAS-TL: O-acetylserine (thiol) lyase; SAT: serine acetyltransferase; SHM: serine hydroxymethyltransferase; GSHA: gamma-glutamylcysteine synthetase; GSHB: glutathione synthetase B; CGS: cystathionine gamma-synthase; CBL: cystathionine beta-lyase; MS: methionine synthase. CK, control; S0, S deficiency; SR, sulfate resupply in both sides of split-roots; SPSR, the split-root halve with sulfate resupply; SPS0, the split-root halve remained in S deficiency. Sh, shoot; R, root.

**A HOLISTIC OVERVIEW OF THE IMPACT OF SULFUR DEFICIENCY IN PEA FACING WATER DEFICIT**

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We report on the interplay between water deficit and sulfur deficiency, two constraints that are increasingly faced by crops due to climate change and low-input agricultural practices. In particular, we aim at better understanding the role of sulfur nutrition in the trade-off between seed quality establishment and plant stress tolerance in pea (*Pisum sativum* L.), a grain legume crop which has a pivotal role to play in both agroecological and food transitions. Like other legumes, pea is able to accumulate large amounts of seed proteins even in the absence of nitrogen fertilizers thanks to its symbiosis with N2-fixing soil bacteria. In this study, we deprived pea plants (cv. Caméor) of sulfur from the mid-vegetative stage and applied a moderate water deficit for 9 days starting at flowering. Individual stresses and control conditions (well-watered, non-limiting sulfur conditions) were applied in parallel for comparison. Phenotypic measurements up to maturity revealed that the combination of the stresses impeded reproductive processes in a synergistic manner, reducing one-seed weight and seed number, thus highlighting the paramount importance of sulfur for maintaining seed yield components when plants encounter a moderate water deficit [1]. Focusing on seed quality attributes, water deficit mitigated the negative effect of sulfur deficiency on the accumulation of sulfur-rich globulins (11S) in mature seeds. Patterns of plant nutrient allocation, including the quantity of sulfur and nitrogen per seed, reflected a lower seed sink strength for nitrogen in the double-stressed plants compared to sulfur-deficient plants. This may readjust the nitrogen to sulfur ratio in the double-stressed seeds, thus rebalancing the amount of 7S (sulfur-poor) and 11S globulins.

To uncover the molecular processes underlying the interplay between the two stresses, seeds and leaves, which are respectively sink and source of nutrients, were collected at different time points before, during and after the double stress period and subjected to multi-omics profiling studies. Inference of a protein network using the seed proteomic data identified a cluster of antioxidant proteins (including a glutathione *S*-transferase, a methionine sulfoxide reductase, and a thioredoxin) that may maintain redox homeostasis in early developing seeds and prevent cellular damage under stress conditions. Integration of these proteomic data with transcriptomic data at the transition to seed filling revealed transcriptional events associated with the accumulation of these antioxidant proteins [2]. This transcriptional defense response mainly involves genes of sulfate homeostasis and assimilation, which is reminiscent to our previous work in Arabidopsis showing that sulfate remobilization in seeds is likely to contribute to the seed's defense against oxidative stress [3]. This study thus provides candidates for targeted studies aimed at dissecting the signaling cascade linking sulfate metabolism to antioxidant processes in developing seeds. These results also highlight the importance of addressing the adaptive mechanisms used by plants to regulate sulfur homeostasis under abiotic constraints. Analysis of the proteomic and transcriptomic data obtained from the leaf samples revealed profound changes in response to water deficit and/or sulfur deficiency, especially when the two stresses were combined. Integration of these data with ionomics data obtained from the same leaf samples, using a weighted-gene co-expression network approach, highlighted genes associated with changes in leaf nutrient concentration, including sulfur concentration. The comparison between leaf transcriptomic and proteomic data allows us to formulate hypotheses on the level of regulation (transcriptional or post-transcriptional) of these genes. We also propose candidate genes that might help plants to better tolerate combinatorial stresses by contributing to adjust nutrient homeostasis in leaves.

Keywords: *Pisum sativum*, sulfur deficiency, water deficit, seed proteins, seed development, leaf metabolism, omics, network.

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**ROLE OF VACUOLAR SULFATE IN NUTRITIONAL QUALITY OF PEA SEEDS**

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Grain legumes have a key role to play in both agroecological and food transitions. Indeed, these plants are able to accumulate large amounts of proteins in their seeds even in the absence of nitrogen fertilization thanks to symbiotic N2 fixation in the root nodules. However, legumes are exposed to abiotic stresses, including nutrient deficiencies, making it important to optimize nutrient use efficiency for maintaining seed protein content and quality. Seed protein quality refers to the ability of the seed proteins to meet the body’s requirements for essential amino acids. It strongly depends on the amino acid balance, which determines protein digestibility. In pea (*Pisum sativum*) seeds, methionine and cysteine are, with tryptophan, the most limiting essential amino acids. Although the literature suggests that sulfate assimilation during seed development may be a source of sulfur amino acids for the synthesis of sulfur-rich storage proteins, work is still needed to understand how sulfate transport and metabolism regulate seed protein composition.

Sulfate ions are transported in plant tissues by sulfate transporters (SULTR) encoded by the *SULTR* gene family. These ions are taken up from the rhizosphere by high-affinity transporters of group 1 (SULTR1) and their root-to-shoot transport is ensured by SULTR2 and SULTR3 transporters, which have also been shown to play a role in the translocation of sulfate within developing siliques or seeds in Arabidopsis. In the sink organs, sulfate can be stored in the vacuoles and remobilized *via* SULTR4 transporters, which allow sulfate efflux for further assimilation. Sulfate assimilation starts by a reaction catalyzed by ATP sulfurylase (ATPS), which produces Adenosine 5’-PhosphoSulfate (APS). APS can be reduced into sulfite ion (SO32-) and sulfide (S2-) by APS reductase (APR) and sulfite reductase (SiR), respectively. Sulfide can be incorporated into *O*-acetylserine (OAS) to provide cysteine, which is the precursor for the synthesis of methionine and glutathione (GSH). Here, we focused on the only gene encoding a SULTR4 transporter in pea to investigate its role in providing sulfate for the synthesis of sulfur-rich storage proteins (2S albumins, 11S globulins) in seeds. Two mutants of this gene were identified through a screening of the TILLING (Targeting Induced Local Lesions IN Genomes) population developed using the pea ‘Caméor’ cultivar. The first mutant, called W/-, has a nonsense mutation at position W78. The second mutant, called E/K, has a missense mutation leading to a substitution of a glutamate to a lysine at position 586 (E586K) in the STAS domain (for Sulfate Transporter and Anti-Sigma antagonist), which is essential for the transport function [1]. These mutants and their corresponding wild-type lines (same genetic background) were phenotyped under sulfur-sufficient and sulfur-deficient conditions.

Seed yield of the *sultr4* mutants was unchanged compared to the wild-type when the plants were cultivated under sulfur sufficiency, suggesting that *sultr4* mutants use sulfate absorbed by roots and/or sulfur metabolites instead of vacuolar sulfate to maintain seed production. Nevertheless, seed yield of *sultr4* mutants was significantly reduced under sulfur deficiency, suggesting a pivotal role for the remobilization of vacuolar sulfate to maintain protein biosynthesis in seeds when external sulfate supply is low. The results also revealed significant changes in seed protein composition of the *sultr4* mutants, even under sulfur sufficiency. In this condition, the relative abundance of the sulfur-rich PA1 albumins was lower in mutant seeds. Moreover, sulfate content of these mature mutant seeds was two times higher than that of wild-type seeds, but seed sulfur content (and sulfur quantity per seed) were unchanged. Altogether, the data uncover a defect in the utilization of vacuolar sulfate within the developing mutant seeds. Based on these findings, we suggest that vacuolar sulfate remobilization during seed development is a vital sulfur source for the synthesis of sulfur-rich 2S albumins in seeds even under ample external sulfur supply.

We studied the kinetics of seed development in the mutant and wild-type lines by measuring the fresh weight, dry weight and water content of seeds collected under sulfur-sufficient conditions. There was no change in these parameters kinetics compared to the wild-type until around 400 degree-days after pollination (that is to say that 400 is cumulative sum of daily mean temperature in °C between pollination day and seed sampling day). This result indicates that both *sultr4* mutants, E/K and W/-, seem to have a normal early seed development. After this stage, water content of the W/- mutant seeds decreased faster than that of wild-type seeds, indicating that a complete loss of function of the SULTR4 transporter accelerates seed maturation. We further investigated the contribution of vacuolar sulfate to seed protein accumulation by focusing on the stages where no change in the seed development kinetics was observed. Wild-type and mutant seeds were harvested at 257 degree-days (embryogenesis) and 312 degree-days (early seed filling). We studied, by qRT-PCR, the expression of genes encoding enzymes of sulfur metabolism and seed storage proteins. For both mutants, the expression of genes involved in sulfate reduction (ATPS1 and APR3) increased during embryogenesis compared to the wild type, whereas the expression of several genes encoding sulfur-rich storage proteins (PA1 and PA2 albumins, and legumin) decreased at the early filling stage. This suggests that vacuolar sulfate availability could regulate storage protein production and consequently influence nutritional seed quality. Based on these data, we present a hypothetical model of the impact of vacuolar sulfate in fine-tuning sulfur metabolism and storage protein synthesis during pea seed development. Further experiments are currently performed to confirm this model.

**Keywords:** *Pisum sativum*, seed quality, vacuolar sulfate, sulfur amino acids, storage proteins

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**SUGAR SIGNALING IS AFFECTED BY SLIM1 DURING SULFUR DEFICIENCY IN *ARABIDOPSIS THALIANA*.**

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The maintenance of major macronutrients’ homeostasis must be strictly controlled, especially when one or more nutrients become limiting in the environment. Sulfur assimilation and glucose signaling are important processes in plant growth and development, as well as stress responses. Despite the fact that these processes are inextricably linked, little is understood about how they exactly affect each other. We discovered that SLIM1, a key transcription factor in sulfur metabolism, is implicated in glucose signaling when sulfur in the environment is limiting. In sulfur deficiency, high concentrations of either mannitol or glucose, as well as sucrose, which is not only a source of glucose but also a signaling molecule, appeared to stimulate the expression of SLIM1-dependent genes. The germination rate of the newly constructed by us CRISPR/Cas9 *slim1\_KO* mutant was severely affected by high glucose and osmotic stress. SLIM1 also positively impacts *PAP1* expression during sulfur deficit by binding to its promoter directly. PAP1 is a well-described positive regulator of anthocyanin biosynthesis in response to sugar and light signaling but also nutrient limitation. In the sulfur deficit conditions, the lack of *PAP1* induction in *slim1\_KO* mutant results in significantly decreased anthocyanin synthesis. Our findings suggest that SLIM1 is implicated in the glucose response during sulfur deficient stress *via* modulating sulfur metabolism and directly affecting *PAP1* expression.

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**Combined biofortification of Broccoli heads with selenium, cysteine, and/or methionine: A potential approach to overcome the antagonistic relationship between sulfur and selenium?**

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In a previous set of experiments on biofortification of broccoli floral heads with selenium, we found that the application of 3 mM Se three times during the reproductive phase, i.e., at the start of the exponential phase, in the middle, and in the end of the sigmoidal growth curve (week 8, 10, and 13 respectively) resulted in a reduction of fresh mass by 39%, along with a reduction of organic S by 10%, whilst Se concentration reached 7 μmol g-1DM. The application of 1.5 mM Se resulted in no reduction of fresh mass, and a reduction of organic S by 9%, with Se concentration at 4 μmol g-1DM. Out of 15 weeks of growth period, the duration of the reproductive phase was 10 weeks, started at week 6.

Towards balanced biofortification, we decided to reduce Se concentration to 0.2 mM and to add cysteine (produced by microbial fermentation), applied foliarly or to soil, to bypass the antagonism between sulfate and selenate. On the other hand, 3 applications are a rather expensive agricultural treatment, therefore we decided to apply once and at the beginning of the exponential phase of sigmoid growth curve.

In this line, several trials were studied by applying sodium selenate 0.2 mM Se once, alone (vs. 1.5 mM, and 3 mM Se), or in combination with cysteine 0.05 mM, and/or methionine 0.10 mM. One hundred and fifty plants were transplanted in pots containing vermiculite, perlite, and sand in a ratio of 1:1:1, in a greenhouse at 2021/10/25 (d0). Once a week, plants were receiving full nutrient solution. Heads appeared on day 60 post transplantation and were harvested on d107. The aforementioned foliar applications took place on d73, i.e., at the beginning of head development, when the exponential growth phase started. Moreover, in another set of plants, foliar application of 0.2 mM Se took place also on d95, i.e., by the end of head development, when the exponential growth phase ceased. A silicon-based wetter (SW7) was used in foliar applications. Selenium, sulfate, and organic sulfur concentrations will be presented, along with chlorophylls and carotenoids contents, and the effect of cysteine will be discussed.

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**EFFECT OF IRON AND GIBBERELLIN INTERPLAY ON SULFUR HOMEOSTASIS OF MAIZE**

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The quest to unravel the mysteries of iron homeostasis has led us to perform an *in silico* analysis of a genetic locus positioned on chromosome 1 in maize, after a preceding study (Benke et al., 2014) pinpointed this locus as a major QTL involved in iron homeostasis. According to Benke and colleagues this QTL, referred to as chrom7-glb1, is related to leaf SPAD values and additionally it seems to control root branching at the terminal 5 centimeters of maize roots (termed as BTR), a phenotype occurring under Fe deprivation and which was further confirmed (Ventouris et al., 2020b). *In silico* analysis revealed that 32 genes are present in chrom7-glb1, two of which are associated with gibberellins (Ventouris et al., 2020a). Zm00001d033379 (GRMZM2G163761) encodes the transcription factor KIP1 (Knotted Interacting Protein 1) which regulates gibberellin biosynthesis, and Zm00001d033396 (GRMZM2G018414) the product of which is known as GRFTF4 (Growth-regulating-factor/GRF-transcription factor 4), a transcription factor implicated in the response to gibberellins. Since the presence of these two genes in chrom7-glb1 implies a tight correlation between gibberellins, the branching in the terminal 5 centimeters of roots, and SPAD values, maize seedlings were grown under iron sufficiency or iron insufficiency and with the addition of gibberellic acid (GA3) or the gibberellin biosynthesis inhibitor Mepiquat chloride. Furthermore, the effect of the interplay between iron and gibberellins on sulfur distribution among shoot and roots was investigated.

In this study, maize seeds (*Zea mays* L., Corteva Agriscience P0937) were placed for germination and on day 6 the seedlings were transferred to hydroponic containers under full nutrition and 100μM Fe. On day 8 after sowing, the seedlings were divided into 4 treatments: plants growing under full nutrition (300μM Fe, treatment: 300), plants growing under iron insufficiency (10μM Fe, treatment: 10), plants growing under full nutrition and in the presence of gibberellins in the nutrient solution (170μM GA3, treatment: 300GA), and finally plants growing under iron insufficiency with the addition of the GA biosynthesis inhibitor Mepiquat chloride in the medium (0,03% v/v, treatment: 10MEP). The containers were placed in growth chambers with a photoperiod of 14h light/ 10h dark, and light intensity equal to 250μmol photons/ m-2 s-1. Seven days after treatment initiation (day 15 after sowing) the shoot and root fresh weight and dry weight were measured, and the total chlorophyll content of every leaf was estimated (Hiscox and Israelstam 1979). Additionally, the shoot and root sulfur (SO42- and organic S) content were assessed (Maniou et al. 2022). The nutrient solutions prepared were identical to these used in Ventouris et al., 2020a, with the exception of the iron content.

As expected under low Fe supply lateral roots appeared at the terminal 5 centimeters of all root types, whereas the roots of seedlings grown in Fe sufficient conditions appeared normal with no branching taking place 5 or less centimeters from the root tips. Interestingly, when GA3 was added to the growth medium of Fe sufficient plants, the BTR phenotype developed irrespectively of Fe supply. This was further supported by the fact that 10MEP plants did not develop the BTR phenotype despite being exposed to low Fe conditions. Besides that, stunted growth of the root system was observed in both the 300GA and 10 treatments, compared to controls (300 treatment) and 10MEP. Regarding the total chlorophyll content, a clear pattern of chlorosis, typical of iron deprivation, was evident in the leaves of 300GA plants. The chlorophyll content of older leaves (1st and 2nd) of both 10 and 300GA plants was comparable to that of 300 plants. However, the younger leaves (leaf 3 and 4) of 300GA seedlings appeared chlorotic and their chlorophyll content did not differ when compared with the leaves 3 and 4 of Fe deprived plants (10 and 10MEP). As expected, the chlorophyll content of leaves 3 and 4 of 300 seedlings was significantly higher compared their 10 and 300GA counterparts, since these plants were grown under Fe sufficiency. Remarkably, all 4 leaves in the 10MEP treatment appeared chlorotic and their chlorophyll content was similar to that of leaves 3 and 4 in both 10 and 300GA plants. Regarding sulfur concentrations, the total S content was significantly higher in 10 and 10MEP plants both in shoots and roots. The total S content of 300GA seedlings was comparable to that of 300 plants in the shoot, but seemed to be higher in the roots of plants treated with GA3. On the other hand, the concentration of organic sulfur was similar in the shoots of all treatments, whereas the organic S content of 300GA roots was significantly higher compared to the rest of the treatments. SO42- concentration was found to be greater in both the shoot and roots of 10 and 10MEP seedlings when compared to 300 and 300GA treatments. Although the SO42- content did not differ significantly between the roots of 300 and 300GA plants, the shoot of 300 seedlings had accumulated larger amounts of SO42- per gram of dry weight than the shoot of maize seedlings treated with GA3 under Fe sufficiency (300GA treatment).

The above results highlight a central role of gibberellins in iron homeostasis, since they are implicated both in the development of the BTR phenotype in roots, as well as in the occurrence of chlorotic symptoms, typical of iron deficiency, in the younger leaves in maize. In addition, the fact that 300GA plants are speculated to sense iron deficiency explains the increased concentrations of organic S observed mainly in the root system, and to a lesser extend in the shoots of these plants. This effect of gibberellins could be related to the two GA-associated transcription factors, the genes of which are found within the chrom7-glb1 locus.

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**NOVEL LINKS BETWEEN ENERGY SENSOR KINASES AND THE ASSIMILATORY SULFUR REDUCTION PATHWAY**

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In contrast to animals, plants respond to environmental challenges with comprehensive developmental transitions to cope with these stresses. The limitation of the soil-borne nutrient sulphur is such kind of environmental challenge causing enhanced root growth to forage the soil for sulphur sources. The promoted root growth causes a substantial increase in the root-to-shoot ratio, which is observed under the limitation of various soil-borne nutrients (e.g., nitrogen, water (Gruber et al., 2013)). These developmental transitions require coordination of apical meristems located in the shoot and roots and substantial redistribution of resources from the shoot to the roots. The sensor kinase Target Of Rapamycin (TOR) is downregulated in the shoots of sulphur starved plants to decrease meristem activity and induce nutrient recycling via self-eating (autophagy, (Dong et al., 2017)). The importance of autophagy for the redistribution of resources from the shoot to seedlings under sulphur limiting conditions has also been shown (Lornac et al., 2020). In a recent study, we demonstrate that maintenance of root TOR activity is critical for the root-to-shoot ratio induction and coordination of autophagy in response to sulphur limitation (Dong et al., under revision). Our data suggest that shoot-specific induction of autophagy and phloem loading of sucrose via SWEET11/12 is essential for the root-to-shoot ratio induction upon sulphur limitation. Remarkably, sucrose translocation from the shoot to the root via SWEET11/12 is also vital for proper root-to-shoot ratio induction upon water limitation (Chen et al., 2022).

However, the total absence of sulphur causes the inactivation of shoot TOR activity in a sucrose-independent manner (Yu et al., 2021). These novel findings open the possibility of multiple regulatory signal transduction mechanisms acting at different sulphur concentrations.

Here, we show initial findings suggesting that the energy sensor kinase SnRK1 (sucrose non-fermenting–related kinase 1) transduces sulphur metabolism-specific signals and sugar-derived signals to TOR via different signal transduction routes operating either in the nucleus or the cytosol. The relevance of these signals for the organ-specific coordination of TOR and autophagy in response to sulphur limiting and sulphur absent conditions will be discussed.

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**The Effects of ReDUCED Methylthioadenosine Recycling are responsive to INCREASED Spermidine Supply**

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Methylthioadenosine nucleosidase (MTN) activity in plants contributes to the regeneration of methionine initiating from methylthioadenosine (MTA) as part of the Yang cycle. Analysis of Arabidopsis mutants deficient in MTN activity revealed that although methionine recycling is not essential for plant growth and development, MTN activity is required. Phenotypic analysis of mutants differing in their residual MTN activity suggests that there is a ‘tipping point’ of MTA accumulation below which plants apparently thrive, whereas further reductions in MTN activity are associated with aberrations in cell division and plant death.

Although Yang cycle enzyme activities were initially thought to be present primarily in phloem cells (1), recent studies show MTN genes are expressed in most cell types, with the transcript abundance being highest in root and shoot apices, and pollen. Thus, a reduction in MTN activity is expected to have differing impacts across various tissues, assuming enzyme activities mimic these transcript levels. Consistent with this prediction, severe deficiency of MTN activity is associated developmental abnormalities in tissues with the highest expression of MTN genes (2). Surprisingly, plants with modest reductions in MTN activity or seedlings grown on media supplemented with low concentrations of MTA have increased growth rates.

To understand these differing effects of MTN deficiency, seed of the most severe MTN deficient mutant were germinated on media supplemented with relevant metabolites. When the medium contained 100 M spermidine (Spd) random branches of the normally sterile mutant became fertile; germination of these seed on Spd-containing media increased seed production in subsequent generations. A molecular evaluation of the effects of MTN deficiency and the impact of Spd treatment revealed MTA accumulates in reproductive tissues and three generations of Spd treatment restore MTA to more normal levels. In addition, Spd treatment restored both the reduced methyl index of the MTN deficient mutants and the general down regulation in the expression of many developmental and reproductive genes.

MTN mutants carrying reporters for auxin or cell cycle regulators reveal that a reduction in sulfur assimilation is associated with mis-regulation of energy metabolism and plant development; Spd treatment attenuates these effects. Together these results suggest a role for Spd in reducing the metabolic impacts associated with sulfur deficiency, and hint that MTA itself may contribute to how plant cells monitor their sulfur availability.

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**A GENETIC APPROACH TO DECIPHER THE IMPACT OF METHYTHIOADENOSINE ACCUMULATION ON PLANT DEVELOPMENT AND SULFUR METABOLISM**

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By coupling photosynthesis with sulfate assimilation into cysteine, methionine, and *S*-adenosylemethionine (SAM), plants finely tune their metabolic activity to adapt their growth and development to the surrounding environment. As part of this process, plants recycle the sulfur-containing by-product of SAM-dependent reactions, methythioadenosine (MTA), using MTA nucleosidase (MTN). *Arabidopsis thaliana* plants with mutations in both MTN genes (*mtn1-1mtn2-1)* have numerous developmental and metabolic abnormalities, including delayed bolting, impaired fertility, fasciation, and reduced polar auxin transport. To better understand the molecular basis of this complex phenotype, a suppressor screen was carried out using the single mutant, *mtn1-1,* which forms a short root when grown on media containing MTA as the sole sulfur source.

Five unique *MTA RESISTANT* (*mtar*) mutants were isolated from ~11,000 EMS-mutagenized M2 *mtn1-1* seeds. Thesemutantsalter genes involved in distinct areas of cell physiology including regulating sulfur flux, meristem maintenance, cell cycle progression, and hormone signaling. The ability of each *mtar* to suppress the phenotypic effects of MTN deficiency was evaluated by crossing each mutation into an *mtn1-1mtn2-1* background. While the expression of reporter genes for meristem activity, cell cycle regulators, and auxin were assessed in *mtn1-1mtar* seedlings grown on MTA. These analyses were complemented by a detailed developmental analysis of each line, including bolting time and fertility assessments. Additionally, cell division, TOR activity, and hormone profiles of these mutants were studied to assess the broader physiological impact of MTA accumulation. A dominant mutant, *mtn1-1mtar11*, was examined in more detail, and it not only provided relief from metabolic pressures of MTA but also increased plant growth and seed production in the double mutant background. Together, our results lead us to propose a role for MTA in the coordination of plant development and sulfur metabolism.

**THE CYSTEINE-RICH 11S GLOBULIN LEGUMIN FROM COMMON BEAN CONTAINS A PEPTIDE RESISTANT TO CLEAVAGE BY PEPSIN (pH ≥ 2) AND CHYMOTRYPSIN**

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The mature form of the 11S globulin legumin from common bean (*Phaseolus vulgaris*) contains five cysteine residues, whereas the 7S globulin phaseolin is devoid of sulfur amino acids. Unlike in soybean and other legumes, 11S globulins are relatively minor constituents of storage proteins in commercial cultivars of common bean (*Phaseolus vulgaris*), with legumin accounting for approximately 3% of total protein. Common bean genotypes lacking phaseolin and major lectins have a substantial increase in sulfur amino acids, particularly cysteine. In such genotypes, legumin becomes the dominant storage protein, accounting for close to 20% of seed proteins. It was previously reported that the 11S globulin, legumin, contains a peptide of ca. 20 kDa which is resistant to simulated gastrointestinal digestion. In grain protein, resistance to proteolytic digestion often correlates with allergenicity. An approach combining purification and mass spectrometry was used for biochemical characterization. Using purified legumin, the peptide of ca. 20 kDa was found to be resistant to pepsin digestion in a pH-dependent manner and was mapped to an internal fragment of approximately 120 amino acid residues near the C-terminal end of the α-subunit. The same fragment coincided with a peptide resistant to chymostrypsin digestion. Results also highlighted the fact that legumin is modified with *O*-glycosylation.

**THE BIOSYNTHESIS OF NON-PROTEIN SULPHUR AMINO ACID IN SEED OF COMMON BEAN**

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The protein quality of common bean (*Phaseolus vulgaris*) is associated with the level of dietary essential sulfur amino acids – methionine and cysteine. Extra sulfur which cannot be stored in the protein pool is accumulated as the non-protein amino acid *S*-methylcysteine (SMC) and its dipeptide γ-glutamyl-*S*-methylcysteine (γ-Glu-SMC) (Taylor et. al., 2008). This study focuses on the biosynthetic pathway of the γ-Glu-SMC and the key intermediate in the biosynthesis of SMC, *S*-methylhomoglutathione (*S*-methylhGSH). One member of the β-substituted alanine synthase (BSAS) family, BSAS4;1, shows a high catalytic activity when acting as a cystine synthase, and catalyzes the formation of free SMC (Joshi et al., 2019). Benzoic acid was identified as an inhibitor for BSAS4;1, and its Ki value was determined to be 0.05 mM. γ-Glutamyl transferase (GGT) is likely to be involved in the biosynthesis of γ-Glu-SMC; its candidate genes were selected from genomic and proteomic databases. The results of sub-cellular localization study indicated that GGT enzyme present in the seed of common bean may accumulate in cytosol, suggesting a possible site of γ-Glu-SMC biosynthesis. Furthermore, the combination of isotope labelling and high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to reveal the mechanism of reactions in this pathway, specifically the reaction from homoglutathione to *S*-methylhGSH. The findings delineate the biosynthetic pathways of the sulfur metabolome of common bean seed and provide an insight that will aid future efforts to improve nutritional quality.

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**TOWARDS INTEGRATED SULFUR-BASED BIOSTIMULATION AND AGRONOMIC BIOFORTIFICATION OF OLIVE TREE PRODUCTION**

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Olive tree (*Olea europaea* L.) is a very important indeterminate long lived, evergreen fruit tree, cultivated mainly in the Mediterranean basin, and experimental evidences have demonstrated both fruits and leaves to be important organs for synthesis of several interesting biological compounds. Olive tree has a plentiful bloom but a low percentage of normal fruit set. (Reale et al., 2006). Inflorescence development is distinguished in three stages (Kitsaki et al., 2012): a first developmental stage corresponding to the elongation of the inflorescence (inflorescence elongation period, IEP), a second one corresponding to the development of the flowers (flower development period, FDP), and the last one corresponding to full bloom (FB) and flower fertilization (flower fertilization period, FFP). The boundary between FDP and FFP (i.e., the week between 2/5 and 5/5/2021 in our experiment) characterized FB.

Towards supporting inflorescence development, the concept of the applied sulfur enhanced fertilization scheme included three steps: (1) the incorporation of elemental sulfur in the first fertilizer application after harvesting of olives (in November), to sustain the next production period, (2) the incorporation of methionine in the 2nd fertilizer application to support the differentiation of floral buds and the current vegetation that will carry the next year’s production, and (3) the application of zinc sulfate, along with boron as ethanolamine borate, molybdate and arginine, as opposed to a conventional fertilization scheme.

1st step: Urea ammonium sulfate (UAS) granules enriched with 3% (w/w) elemental sulfur (ES) plus 1% N-(n-butyl) thiophosphoric triamide (NBPT) via a binder. After its application to soil, U undergoes hydrolysis via the urease enzyme, thus resulting in ammonia losses due to pH increase in the soil volume surrounding the granules. The use of urease inhibitors is an effective way to reduce ammonia losses. The most commonly used one is NBPT. The coexistence of sulfate and ES, along with NBPT, in fertilization schemes has been discussed (Bouranis and Chorianopoulou, 2018).

2nd step: A liquid fertilizer was prepared and applied foliarly, containing nitrogen 5% w/w in two forms: ammonium 2,4% w/w and urea 2,6% w/w, phosphorus 20% w/w and L- methionine (Met) 5% w/w. In addition to its participation as a structural component of proteins, Met serves as the precursor of sulfur-adenosyl-methionine (SAM), a key metabolite. SAM feeds various metabolic pathways, one of which is the production of nicotianamine (NA), a chelator. SAM is a methyl donor in many metabolic pathways and therefore contributes to dealing with adverse crop conditions in various levels, thus supporting the crop to cope effectively with the adverse conditions.

3rd step: A liquid fertilizer was prepared and applied foliarly, containing boron (B) 5% w/w in the form of ethanolamine borate, zinc (Zn) 2% w/w as zinc sulfate, molybdenum (Mo) 1% w/w and L-arginine 5% w/w (Arg). The fertilizer supports the crop during the reproductive stage, for optimal flowering and fruiting. Reproductive phase is particularly sensitive to boron deficiency that causes significant losses in production, even though no visible deficiency symptoms are observed in the vegetative phase. Boron deficiency is often responsible for reduced fruit setting. Foliar application with boron prior to flowering results in increased yields even when elemental analysis of soil and plant tissues shows sufficient boron levels because it contributes to the alleviation of potential existence of transient however critical deficiency during the reproductive phase. Zinc is needed in small quantities and contributes to the normal performance of many functions within the plant. These functions play important role in the growth regulation, enzyme activation, phytohormone activity, photosynthesis, protein synthesis, carbohydrate metabolism, reproductive phase, seed production and protection against illnesses. In addition, resistance to adverse environmental conditions on crops, is associated with a high demand for zinc. Agronomic biofortification schemes usually include zinc sulfate. Molybdenum participates in various plant functions and the most important contribution is in nitrogen metabolism. Moderate or latent molybdenum deficiency limits production because of combined nutrition disorder with nitrogen and sulfur, especially if they occur in the reproductive phase. This micronutrient package is supported by the amino acid Arg, which is an organic source of nitrogen. Arginine metabolism not only plays a key role in the distribution and recycling of nitrogen in cultivated plants, but also it is a precursor towards producing reactive nitrogen and polyamines, which in turn support the crop to encounter with adverse situations by activating defense mechanisms, especially during flowering. The 3rd fertilizer also enhances the crop performance with ethanolamine along with boron. Plants synthesize ethanolamine by decarboxylation of serine, and they produce choline, a molecule of special importance. It is a methyl donor but also a precursor of glycine-betaine, an osmosis-protective molecule. Choline is needed for the synthesis of phosphatidylcholine associated with the stability and more efficient function of membranes. Enhancing the plant with choline can therefore contribute substantially to its resistance to adverse conditions such as drought and salinity. Choline is usually produced in small quantities and the plant needs to be strengthened; higher choline levels contribute to production of a higher quality. A silicon-based wetter was used in foliar applications.

Nine, 48 years old, olive trees of an olive tree orchard in Flomochori village, Laconia, Greece, received the conventional scheme, while other nine trees received the presented fertilization scheme, which provided an increase in fruit production by 38%, during the agricultural period of December 2020 – November 2021 (first year). Olive oil produced under this approach was of superior quality. The experiment is ongoing, running now in the second year. Application details and timing will be discussed, along with the preliminary results at hand so far. The applied scheme, that integrates a sulfur-based biostimulation approach along with an agronomic biofortification one, seems to be a successful fertilization scheme for both the olive fruit and the olive oil production.

**Acknowledgments**: Karvelas AVEE provided all fertilizer ingredients; the used amino acids are fermentation products.

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EFFECTS OF EXCESS SULPHUR ON CADMIUM UPTAKE AND TRANSLOCATION IN SOYBEAN

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Cadmium (Cd) is a non-essential element that can inhibit plant growth. If it accumulates in edible crops, Cd poses a health risk to consumers. Cadmium concentrations are increasing globally in agricultural soils due to anthropogenic contamination (Peralta-Videa et al., 2009). Plants have two primary mechanisms to reduce metal toxicity to roots: (1) the formation of plaques (layers of oxidized compounds on the root surface, primarily iron-hydroxides), which form insoluble complexes with metals and prevent them from entering the plant (Seregin and Ivanov, 2000); (2) the production of chelators, including thiol-containing phytochelatins, which form complexes with metal ions (especially Cd2+) that are transported into vacuoles where the metal ions remain sequestered; thereby, reducing Cd translocation from the roots to the shoots (Seregin and Ivanov, 2000; Li et al., 2017). In soybean (*Glycine max*), these chelator compounds primarily include homophytochelatin (hPyC2) and homoglutathione (hGSH) as well as gamma-glutamyl-cysteine, glutathione, and phytochelatin (PyC2). Another method for plants to reduce Cd toxicity is through binding of Cd to cell walls, which prevents the movement of Cd into cells and through surrounding plant structure.

Sulphur (S) addition to Cd-contaminated soils has been shown to reduce Cd toxicity in rice (Li et al., 2017) and wheat (Chen et al., 2007). Proposed mechanisms for this phenomenon include S-induced formation of an insoluble plaque on the root surface, which prevents Cd uptake into the roots, and/or increased S availability may subsequently increase the production of chelators that bind to and sequester Cd within plant cells.

The aim of this study was to determine the underlying mechanisms behind reduction of Cd toxicity due to S addition in soybean. The specific objectives were to determine if S addition will reduce Cd toxicity by causing increased binding of Cd to the root plaque, decreased Cd uptake, increased chelator production, and increased sequestration of Cd in vacuoles. Hydroponic and soil experiments were performed to determine the potential for excess S addition to reduce Cd toxicity.

In contrast to results of similar experiments on rice (Li et al., 2017) and wheat (Chen et al., 2007), this study has shown that excess S does not reduce Cd toxicity in soybean. Addition of excess S did not reduce the concentration of Cd in roots or shoots compared to plants grown with no additional, nor did it increase plant growth in the presence of Cd. Excess S did visibly increase plaque formation on the root surfaces, but the concentration of Cd bound to root plaques has yet to be determined. Excess S did not increase vacuolar sequestration in the root cortical cells but did increase Cd localization in the stele. As expected, concentrations of chelators in roots and leaves increased in response to Cd. The chelators in order of increasing abundance were gamma-glutamyl-cysteine < hGSH < hPyC2. Concentrations of hPyC2 increased at least 10-fold in response to Cd for plants grown hydroponically (data not shown) and in soil (Figure 1). Excess S resulted in a 0 to 4-fold increase in gamma-glutamyl-cysteine and hGSH across all treatments in hydroponic- and soil-grown plants (data not shown) but the pattern was not consistent with S dose. Excess S resulted in 30% higher concentration of hPyC2 in roots grown in soil spiked with 400 mg Cd per kg of soil (Figure 1b).

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| Figure 1. Concentrations of homophytochelatin (hPyC2). Concentrations per mg dry weight (DW) were measured for A) leaves and B) roots of soil-grown plants treated with either 0, 1, 5, or 10 mM added Na2SO4 and 0, 300, or 400 mg of CdCl2 per kg of soil. Different letters indicate a significant difference of hPyC2 concentration, as determined by a two-way ANOVA followed by post-hoc Tukey tests (P < 0.05), with n = 3. Values plotted are mean ± SE. Statistical analysis and graphing were performed using SigmaPlot11. | |

Based on the findings of this study it can be concluded that addition of excess S in the presence of Cd does not reduce toxicity of Cd in soybean as it does in rice and wheat. These results would best be applied by agronomists to prevent misconceptions about the benefits of excess S on reducing Cd uptake and toxicity in crop species such as soybean.

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**INTERACTIVE EFFECT OF NITROGEN AND SULFUR SUPPLY ON YIELD AND RELATED TRAITS OF CANOLA**

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Rapeseed is sensitive to sulphur (S) nutrients and profitability of canola production depends on an adequate and balanced supply of nitrogen (N) and S fertilizers. A field study was conducted to test the effects of different N and S fertilizer combinations on plant tissue S concentration, S and N uptake, yield, and seed oil and protein under different soil textures for 3 growing seasons. Our results showed signs of S deficiency (cup-shaped leaves and purple leaf margins) on clay loam for at least two growing seasons. In 2021, although high pre-plant soil sulfate results were measured, signs of S deficiency were also observed in some plots without N fertilization. Both N and S fertilizer supplies increased seed yield, and their interaction occurred in only 2 of the 12 sites-years. Canola yield was positively correlated with pods plant-1 in 10 of the 12 site-years. Plant heights, branches plant-1, seeds pod-1, pods m-2 and seeds m-2 were all strongly positively correlated with yield for at least 7 site-years. The average calculated most economic rate of sulfur (MERS) for eastern Ontario and western Quebec is 28.8 kg ha-1 producing an average yield of 2550 kg ha-1. The impact of heat and drought stress on yield was significantly different between 2020 and 2021, indicating the challenges and opportunities for stress mitigation through the development of new integrated agronomic solutions.

**UNDERSTANDING THE MOLECULAR MECHANISMS MEDIATING THE CROSSTALK BETWEEN IRON AND SULFUR NETWORKS IN PLANTS.**

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If given enough water and light, plants can assimilate all the nutrients they need in elemental or inorganic forms (e.g. Fe2+, SO42-) and synthesize all the molecules required to complete their life cycle. Biochemically speaking, this is a feat that only few organisms on Earth can achieve. Plants however, also need to regulate the uptake of nutrients to prevent an overload. This is particularly critical for reactive elements such as iron (Fe), which is essential for respiration and photosynthesis but in excess, promotes the formation of reactive oxygen species (ROS), which may damage proteins, membranes, and DNA. Sulfur metabolism in plants is tightly associated with Fe homeostasis; this may not be surprising considering that iron-sulfur (Fe-S) clusters are at the core of respiratory and photosynthetic complexes. However, how these two pathways communicate with each other at the molecular level is unknown.

We have recently discovered that the primary site of Fe sensing in plants is the leaf vasculature, which prompted us to pursue whole genome transcriptome analyses in leaves in response to Fe deficiency at relatively short periods of time (0-15 hr). Perhaps one of the most exciting results of this time-series analysis, was the discovery that genes associated with sulfur metabolism, including transport and reduction are tightly correlated with Fe deficiency responses. Of particular interest was AtNEET, a 2Fe-2S protein found to be among the fastest de-regulated genes in leaves when Fe becomes limiting. AtNEET belongs to a family of proteins with a unique structure, where 2Fe-2S clusters are coordinated by 3 Cys and 1 His. To further characterize AtNEET, we generated transgenic plants expressing a variant where the single His coordinating the Fe-S clusters was mutated to Cys, making the Fe-S clusters remarkably stable and less prone to be transferred between proteins. Interestingly, plants expressing this AtNEET variant (H89C) display severe developmental phenotypes and a constitutive Fe deficiency response. At the meeting we will discuss a model that places AtNEET at the crossroad between Fe sensing, sulfur metabolism, and redox control in plants.

TRACKING DYNAMIC CHANGES OF LEAVES IN RESPONSE TO NUTRIENT AVAILABILITY USING AN OPEN-SOURCE CLOUD-BASED PHENOTYPING SYSTEM (OPEN LEAF)

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Micronutrients, such as iron, zinc, and sulfur, play a vital role in both plant and human development. Understanding how plants sense and allocate nutrients within their tissues may offer different venues to develop plants with high nutritional value. Despite decades of intensive research, more than 40% of genes in Arabidopsis remain uncharacterized or have no assigned function. While several resources such as mutant populations or diversity panels offer the possibility to identify genes critical for plant nutrition, the ability to consistently track and assess plant growth in an automated, unbiased way is still a major limitation. High-throughput phenotyping (HTP) is the new standard in plant biology, but few HTP systems are open source and user friendly. Therefore, we developed OPEN Leaf, an open source HTP for hydroponic experiments. OPEN Leaf is capable of tracking changes in both size and color of the whole plant and specific regions of the rosette. We have also integrated communication platforms (Slack) and cloud services (CyVerse) to facilitate user communication, collaboration, data storage, and analysis in real time. As a proof-of-concept, we report the ability of OPEN Leaf to track changes in size and color when wildtype are grown hydroponically with different levels of nutrients and the mutants slim1-1/2 under sulfur deficiency. We expect that the availability of open source HTP platforms, together with standardized experimental conditions agreed by the scientific community, will advance the identification of genes and networks mediating nutrient uptake and allocation in plants.

**Sulfur in plant responses to cadmium stress: from signaling to acclimation**

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The environment of plants is ever-changing and as sessile organisms, plants are prone to environmental challenges, like cadmium (Cd) pollution. Importantly, stress perception and its downstream responses should be considered as context-dependent, and are influenced by the stress type, severity, and duration. At the cellular level, Cd stress modifies the balance between production and scavenging of reactive oxygen species (ROS). Nevertheless, maintaining this oxidative balance is crucial for plant stress acclimation. Therefore, a central role for the antioxidant glutathione (GSH) is put forward in the acclimation of plants to Cd stress, because it is involved in neutralizing ROS but also serves as a precursor for phytochelatins (PC) chelating and sequestering Cd ions. Glutathione also plays an important role in the functioning of the endoplasmic recticulum (ER). The oxidising environment in the ER is crucial for the stabilisation of the formed disulfides, which are indispensable for proper protein function. An integral aspect of stress is the accumulation of unfolded or misfolded proteins (i.e., proteotoxic stress) in the ER and other organelles, which triggers an unfolded protein response to mitigate ER stress. This intracellular signalling mechanism aims to restore protein homeostasis by upregulating genes involved in protein folding and ER-associated degradation, or by induction of autophagy. The latter aims to recycle damaged cellular components by engulfing them in vesicles (i.e. autophagosomes) and transporting them into the vacuole for nutrient recycling. Autophagy is increasingly put forward as a protective mechanism during various stresses that may be intertwined with the redox environment.

In this study on Cd acclimation responses, giving attention to Cd-induced ER stress and autophagy, acute responses (0-24 h) to Cd exposure (0-5 µM) were investigated to address signal transduction and identify the pressure points (initial responses) of Cd stress in *Arabidopsis thaliana*. A prolonged Cd exposure (72 h) was included to obtain a first indication of acclimation, as a new steady state is typically established at this time point.

The rapid depletion of root GSH concentrations upon Cd exposure (0-5 µM) is proposed to serve as an alert response in *A. thaliana*. During this alarm phase no changes in the GSH redox state were observed and we suggest that a strong depletion in GSH concentrations is sufficient to alter the cell’s redox potential and drive acclimation responses. Ethylene is a known mediator of the Cd-induced oxidative challenge and is closely intertwined with GSH. Investigating the reciprocal interaction between both components after Cd exposure revealed an accelerated production of the ethylene precursor ACC and ethylene signalling in GSH-deficient mutants, whereas a delayed GSH recovery was observed in mutants defective in ethylene signalling and ACC biosynthesis as compared to WT plants.

The interdependence between GSH and ethylene with oxidative challenge as a possible mediator is essential in plant acclimation responses to Cd stress.