The phenotypes of Arabipsis mutants for the group 3 sulphate transporters point diverse roles within developing seed

Hélène Zuber, Jean-Claude Davidian, Markus Wirtz, Rüdiger Hell, Maya Belghazi, Richard Thompson, Karine Gallardo

2nd SULPHYTON Meeting

on Plant Sulfur Research

13th - 15th September 2009

John Innes Centre Norwich

Dear Colleagues and Friends

I am happy to welcome you to the 2nd Sulphyton meeting on Plant Sulfur Research in Norwich.

Like its predecessor in Potsdam, this meeting should be a friendly and informal opportunity to present and discuss the latest developments in broad range of areas of sulfur research but even more importantly, a chance to meet friends and colleagues.

I hope you enjoy the meeting and your stay in Norwich.

Local organizing team:

Stan Kopriva
Helen Ghirardello
Anna Koprivova
Colette Matthewman
Nicola Hockin
Michal Bochenek

PROGRAMME

Sunday 13th September

14:00 - 14:55 Registration and Coffee

1st Session chair Stan Kopriva

14:55 **Stan Kopriva,** *JIC Norwich*
Welcome and Introduction

15:00 **Cornelia Herschbach,** *University of Freiburg*
Sulfur flux through the sulfate assimilation pathway is differently controlled by adenosine 5'-phosphosulfate reductase under stress and in transgenic poplar plants

15:25 **Hélène Zuber,** *INRA Dijon*
The phenotypes of Arabidopsis mutants for the group 3 sulphate transporters point diverse roles within developing seed

15:35 **Naoko Yoshimoto,** *Chiba University, Japan*
Functional characterization of ATP sulfurylase gene family in A*rabidopsis thaliana*

16:00 - 16:30 Tea and Coffee

2nd Session chair Malcolm Hawkesford

16:30 **Colette Mathewmann,** *JIC Norwich*
ATP sulfurylase is part of glucosinolate biosynthesis network in Arabidopsis

16:40 **Sarah Mugford,** *JIC Norwich*
APS kinase is essential for Arabidopsis viability

16:50 **Markus Wirtz,** *Heidelberg Institute for Plant Sciences*
Significance of Sulfite Reductase in the Higher Plant *Arabidopsis thaliana*

17:15 **Joseph M. Jez,** *Donald Danforth Plant Science Center St. Louis*
Assembly of the cysteine synthase complex and the regulatory role of protein-protein interactions

17:40 **Silvia A. Tavares,** *Instituto Superior Agronomia Lisbon*
Serine acetyltransferase family in *Vitis vinifera*; the peculiarity of vvsat7

17:50 **Hannah Birke,** *Heidelberg Institute for Plant Sciences*
Evolutionary Aspects of Cysteine Synthesis

18:00 Drinks and Hot Fork Buffet

Monday 14th September

3rd session chair Jean-Claude Davidian

9:00 **Anna Koprivova,** *JIC Norwich*
Genetic dissection of demand-driven regulation of sulfate assimilation

9:25 **Bok-Rye Lee,** *JIC Norwich*
Interaction of glutathione with flowering time

9:35 **Hans-Michael Hubberten,** *MPI-MP Golm*
Identifying "regulatory elements" of sulfur homeostasis

9:45 **Holger Hesse,** *MPI-MP Golm*
Acclimation to stress and assessment of the 'metabolome' compartmentation in Arabidopsis

10:10 **Corinna Hermsen,** *University of Halle*
Is the regulation of sulphate assimilation in *P. patens* like in higher plants?

10:20 **Mutsumi Watanabe,** *MPI-MP Golm*
Regulation of leaf senescence in *old3* mutant of *Arabidopsis*

10:30 - 11:00 Coffee and Tea

Plenary lecture

11:00 Fabien Chardon, *INRA Versailles*
Deciphering complex metabolisms by exploring natural diversity in *Arabidopsis thaliana*

12:00 - 14:00 Lunch

4th Session chair Rainer Höfgen

14:00 **Dimitris L. Bouranis,** *Agricultural University of Athens*
Nitrogen dynamics in maize at the seedling stage under S-deprivation

14:25 **Katarzyna Zientara,** *Institute of Biochemistry and Biophysics Warsaw*
UP15 protein and its potential role in coordination of S and N metabolism

14:50 **Sara Amâncio,** *Instituto Superior Agronomia Lisbon*
Vitis vinifera secondary metabolism as affected by sulfate depletion: diagnosis through phenylpropanoid pathway genes

15:00 **Anne Honsel,** *University of Freiburg*
Characterization of early changes in gene expression of leaves of different developmental stages under sulphur deficiency conditions in poplars (*Populus tremula x P. alba*)

15:10 **Grzegorz Moniuszko,** *Institute of Biochemistry and Biophysics Warsaw*
Impact of sulfur deficiency and hormone treatment on tobacco seedlings roots.

15:20 **Bernd Zechmann,** *University of Graz*
Effects of sulfur treatment on glutathione contents and disease development in TMV-infected tobacco plants

15:45 **Dirk Wesenberg,** *University of Halle*
Quantification of thiols at femtomolar level using laser-assisted microdissected fungal and plant material

16:00-16:30 Tea and Coffee

5th Session chair Rudiger Hell

16:30 **Gill Malin,** *University of East Anglia Norwich*
The significance of sulphur in marine biogeochemistry

16:55 **Nicola Hockin,** *University of East Anglia Norwich*
A Proteomic Approach to Understanding DSMP Production in a Marine Diatom

17:05 **Mariusz A. Bromke,** *MPI-MP Golm*
Metabolite profiling of the adaptive response to temporarily and nutrient dependent changes of the metabolism of *Thalassiosira pseudonana*

17:15 **Michal Bochenek,** *University of East Anglia Norwich*
What controls DMSP synthesis in Emiliania huxleyi ?

17:25 **Tamas Dalmay,** *University of East Anglia Norwich*
Regulation of sulphur assimilation by microRNA-395

18:00 Dinner

Tuesday 15th September

6th Session chair Karine Gallardo

9:00 **David E. Salt,** *Purdue University West Lafayette*
Differential regulation of serine acetyltransferase is involved in nickel hyperaccumulation in *Thlaspi goesingense*

9:25 **Luit J. De Kok,** *University of Groningen*
Supra-optimal copper levels may disturb the regulation of uptake and metabolism of sulfate in plants

9:50 **Muhammad Shahbaz,** *University of Groningen*
Interaction between atmospheric and pedospheric sulfur nutrition and copper toxicity in chinese cabbage

10:00 **Anja Bräutigam,** *University of Halle*
Chlamydomonas synthesises several phytochelatin isoforms upon Cd-stress

10:10 **Emanuelle Cabannes,** *Rothamsted Research*
Sulphate/selenate transporters in selenium hyper-accumulating plants

10:20 **Tamara Gigolashvili**, *University of Cologne*
Identification of plastidic transporter involved in the biosynthesis of Met-derived glucosinolates in *A.thaliana.*

10:30-11:00 Coffee and Tea

7th Session chair Peter Buchner

11:00 **František Zelený,** *Research Institute of Crop production Prague*
Effect of nitrogen and sulfur fertilisation on growth of plants, health, yield, lycopene content and colour of tomato fruits

11:10 **Silvia Haneklaus,** *Institute for Crop and Soil Science Braunschweig*
Influence of sulphur form and rate on growth of phytopathogenic fungi

11:20 **Eva Zelená,** *Research Institute of Crop production Prague*
Effect of sulfur nutrition on glutathione content in sugar beet plants in relation with aphids infestation

11:30 **Ashley Houlden,** *University of Manchester*
Microbial arylsulfatases: diversity and activity in the environment.

11:40 **Achim Schmalenberger,** *University of Sheffield*
The role of sulfonate desulfurizing *Polaromonas* sp. in sulfate limited soils

12:00 Lunch and Departure

ABSTRACTS

Vitis vinifera secondary metabolism as affected by sulfate depletion: diagnosis through phenylpropanoid pathway genes

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Grapevine (*Vitis vinifera* L.) is rich in phenylpropanoid compounds, including polyphenols (flavonoids and stilbenes). The stilbene resveratrol and their derivatives enhance plant resistance to biotic stress and were classified as phytoalexins. Nutrient stress, the lack of nitrogen, phosphorus or sulphur, can bring about the accumulation of polyphenols [1]. This result is explained by the carbon/nutrient balance hypothesis: growth at the expense of C from secondary metabolites. So, as working hypothesis, S deficiency restrains the demand for basic C-compounds and up-regulates the secondary metabolism pathway gene expression. Grapevine is an excellent model to test that hypothesis considering its major investment in the polyphenol pathway.
Chalcone synthase (CHS) and stilbene synthase (STS) are the branching enzymes of the phenypropanoid biosynthetic pathway, CHS for the synthesis of chalcone, the flavonoids precursor; STS for the synthesis of stilbenes, e.g. resveratrol. The downstream flavonoid biosynthesis includes dihydroflavonol reductase, anthocyanidin synthase, UDP-glucose:flavonoid 3-*O*-glucosyltransferase and flavonoid 3',5'- hydroxylase. Sequences from databases confirmed after grapevine genome release [2], [3] were used to analyse gene expression for the enzymes referred above in response to S starvation, in chlorophyll tissues and non-chlorophyll cells. The results will be discussed in function of the biological system.

Acknowledgements
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References:
[1] Nikiforova *et al.* J Exp. Bot. 55, 1861-1870 (2004)
[2] O. Jaillon, J.O. Aury, B. Noel, *et al.* Nature 449, 463 (2007);
[3] R. Velasco, A. Zharkikh, M. Troggio, D.A. Cartwright, A. Cestaro, *et al.* PLoS ONE, 2, e1326 (2007)

Evolutionary Aspects of Cysteine Synthesis

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In the bacterial and plant kingdoms *de novo* cysteine synthesis represents the final step of assimilatory sulfate reduction. Cysteine is produced in a two-step reaction from acetyl-CoA, serine, and sulfide by serine acetyltransferase (SAT) and O-acetylserine(thiol)lyase (OAS-TL). SAT and OAS-TL together form the cysteine synthase complex (CSC). Although the crystal structures of SAT and OAS-TL in *Escherichia coli* and *Haemophilus influenza* are resolved, the exact quaternary structure of the CSC is not known so far. However, structural investigations suggest a decameric complex composed of one SAT hexamer and two OAS-TL dimers. Strong conservation of the amino acid sequences of both the SAT and OAS-TL suggests a similar structure for the subunits and the complex in plants. In fact, *in vivo* size exclusion chromatography experiments support this hypothesis for *Arabidopsis thaliana*. Nevertheless, functional differences occur in bacteria and plants: Whereas cysteine synthesis is regulated *via* a cysteine regulon in *E. coli*, complex formation constitutes the basis for regulation in *A. thaliana*: Whereas SAT is catalytically active within the complex, OAS-TL is only active in its free form. Until now, the reason for the different functions of CSC formation in bacteria and plants is unknown. Investigations of the quaternary structure of the CSC in the non-vascular plant *Physcomitrella patens* and the monocot *Oryza sativa* are on the way and will complement our current knowledge about the CSC.

What controls DMSP synthesis in *Emiliania huxleyi*?

Michal Bochenek[1], Thomas Bell[1], Stanislav Kopriva[2] and Gill Malin[1]

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Dimethylsulphoniopropionate (DMSP), which is synthesised by many marine algae, is the major precursor of dimethylsulphide (DMS) – a sulphur trace gas hypothesised to drive a feedback loop between plankton and climate. Despite the broad and extensive research on DMS and DMSP, the pathway between sulphate uptake and DMS synthesis is not fully characterised. The overall aims of our project are to advance knowledge of the controls on DMSP synthesis and breakdown by the coccolithophore *Emiliania huxleyi* CCMP 1516 using biochemical and molecular tools. This single-celled phytoplankton is a widespread and important DMSP producer which is thought to play a vital role in the global sulphur cycle. In addition, the genome sequence of this isolate has recently been made available. As an initial step we have evaluated the importance of sulphur for cell growth and DMSP synthesis. *E. huxleyi* cultures were grown in media with varying sulphate concentrations but no variation in ionic strength. The results demonstrate that cell growth rate and intracellular DMSP concentration decreases under low sulphate conditions. Addition of various forms of sulphur (sulphate, methionine and cysteine) back to sulphate-limited cultures altered the growth rate and DMSP production in different ways. These data and the genome sequence provides us with the basis for further physiological and gene expression studies. Our current objective is to use Illumina mRNA sequencing to identify genes regulated by sulphate availability.

Nitrogen dynamics in maize at the seedling stage under S-deprivation

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The period of the first 20 days of a maize seedling is crucial for its establishment and growth. In the absence of external nutrient feed for 30 days, the seedling followed a concrete N management programme. The Cisko seed contained 286±7 μmol N, 87.4% of which was transportable to the growing seedling. The seed emptied within three weeks. Root N increased for the first 2 weeks, accumulating the 23% of the available seed N, and stabilised thereafter. Nitrogen began to empty from the primary and seminal embryonic root after the 1st and 2nd week respectively, while the nodal (postembryonic) root increased N amount undisturbed. The aerial part accumulated N for the first 1.5 week, whilst after the 3rd week a decrease was observed. Stem+sheaths N-level remained almost stable. The 4th week was characterised by N export to the rhizosphere (pure water).
We established S-deprivation 1 week and 2 weeks after seed germination and we studied the N accumulation within organs. N-level of leaves clearly diminished almost from the beginning of the deprivation. The N-level in the root system was the same as control after a week in S-deprivation and then it decreased. S-deprived primary roots increased N-levels in all sectors after 3 days onwards. Seminal roots invested in their apex and emerging lateral root sectors from the beginning, while S-deprived 1st nodal roots started to possess less N after 3 days of deprivation. The N-level in the stem+sheaths was slightly lower compared with control during the 2nd week, indicating that the plant clearly makes efforts to maintain the N-levels of this part, probably because it contains shoot's growing point.

Chlamydomonas synthesises several phytochelatin isoforms upon Cd-stress

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Phytochelatins are non ribosomal synthesized peptides with the general structure $(\gamma\text{-GluCys})_n$Gly. According to the literature the amount of γ-GluCys units can differ from 2-11. The fact that *Chlamydomonas reinhardtii* synthesises phytochelatins (PCs) after metal exposition was described already in 1988. But until now no unambiguous sequence analysis of the PCs has been reported. The aim of this study was to identify all PCs synthesised by the alga. Extracts of cadmium-exposed *C. reinhardtii* cells were analysed by ESI-QTOF-MS/MS. Special emphasis was made on reduction of PCs prior to analysis, because oxidation is one of the most frequent source of error in thiol analysis concerning the quantification as well as the identification. As a result, canonic PCs with up to five γ-GluCys-units were detected. Phytochelatin-isoforms Cys_nPC and PC_2Ala were observed for the first time in this model organism. Whereby CysPCs seem to be the major portion of synthesised PCs. Additionally $CysPC_ndesGly$, $PC_ndesGly$, $CysPC_nGlu$, and PC_2Glu were found throughout MS analysis.

Metabolite profiling of the adaptive response to temporarily and nutrient dependent changes of the metabolism of *Thalassiosira pseudonana*

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Diatoms are eukaryotic, photosynthetic microorganisms being responsible for as much as 20% of global primary productivity. Marine primary productivity is regulated by the availability of nutrients. Although a number of factors such as iron and silica are known to influence marine productivity knowledge about mechanisms and adaptation of cellular metabolism are limited. In a non-targeted approach we started to explore biochemical strategies preferred by *Thalassiosira pseudonana* to adapt to different nutrient availabilities on metabolic and molecular levels. We describe metabolic the flow of nutrients in relation to the sulfur metabolism, which is greatly dependent on carbon availability. Tight interactions between these pathways may influence biogeochemical cycling of elements and bring visible consequences in environment. Moreover, the available sequence information of *Thalassiosira pseudonana* genome provides additional information for interpreting the metabolomic data. Sequence analysis and comparison to other species allows us to draw putative metabolic pathway models.

Sulphate/selenate transporters in selenium hyper-accumulating plants

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Selenium (Se) is an essential micronutrient for animals. Selenium deficiency in the human diet is associated with health disorders including cancer and thyroid (Hawkesford and Zhao, 2007). In the UK, wheat is an important source of bioavailable selenium. The ability of some plants to hyper-accumulate selenium can be used to better understand selenium uptake and subsequently to develop breeding strategies for improved Se accumulation in crop species. Plants take up selenium as selenate from the soil. Selenate and sulphate are thought to be transported by the same proteins and to compete in the uptake process. The gene family of sulphate transporters is subdivided into five groups with distinct expression and/or functional characteristics (Hawkesford, 2003).
To investigate the role of sulphate transporters in selenate uptake, cDNAs for sulphate transporters were cloned from both selenium hyper-accumulating *Astragalus* species and closely related non-accumulating *Astragalus* species. Sequence variation has the potential to modify the ratio of selectivity of sulphate and selenate transport. High affinity Group 1 and low affinity Group 2 *Astragalus* transporters were further cloned in a yeast vector and transformed into a yeast mutant deficient in sulphate transport ability to facilitate functional analysis. A hydroponic system has been set up and different treatments with or without sulphate and/or selenate were applied. Expression patterns *in planta* were obtained by semi-quantitative polymerase chain reaction for four groups of sulphate transporters. Ion analyses showed that sulphur starvation increases selenium accumulation in shoot tissues, and selenate treatment increases sulphate accumulation in shoot tissues.

References:
Hawkesford MJ (2003) Transporter gene families in plants: the sulphate transporter gene family — redundancy or specialization? Physiol. Plant. 117: 155-165.
Hawkesford MJ, Zhao FJ (2007) Strategies for increasing the selenium content of wheat. J. Cereal Sci. 46: 282-292.

Deciphering complex metabolisms by exploring natural diversity in *Arabidopsis thaliana*

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Plant growth and development ultimately depends upon environmental factors, such as temperature, light intensity, availability of water and essential minerals. Improving plant mineral use efficiency or controlling soil minerals requires a better knowledge of the regulation of plant metabolism. This could be achieved using *Arabidopsis thaliana* as a model genetic system, and taking advantage of the natural variation available among ecotypes. In our experiments, we investigated sulphur and nitrogen metabolisms either directly by taking the mineral content in plant as a complex trait or by analysing the genetic response to the mineral availability. For example, we show that variation in sulphate content between wild accessions Bay-0 and Shahdara is controlled by a major quantitative trait locus that results in a strong interaction with nitrogen availability in the soil. Using a candidate gene approach, we have cloned the underlying gene, showing how a single–amino acid substitution in APR2, a key enzyme of the assimilatory sulfate reduction pathway, is responsible for a decrease in enzyme activity, leading to sulfate accumulation in the plant. In a second approach, we studied natural variation in plant response to varying nitrogen nutritions within a core-collection of 24 accessions. Following their adaptation to nitrogen limited supply and nitrogen stress condition; we distinguished three classes of responses. Accessions belonging to three classes showed interesting phenotypic variation which can be exploited to identify genes and alleles important for nitrogen metabolism and nitrogen use efficiency.

Regulation of sulphur assimilation by microRNA-395

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Plants play an important role in the global sulphur cycle because they assimilate sulphur from the environment and build it into methionine and cysteine. Several genes of the sulphur assimilation pathway are regulated by microRNA-395 (miR395) that is itself induced by low-sulphur (-S) environment. Here, we show that the six *Arabidopsis* miR395 loci are induced differently. We find that MIR395 loci are expressed in the vascular system of roots and leaves and root tips. Induction of miR395 by –S environment in both roots and leaves suggests that translocation of miR395 from leaves to roots through the phloem is not necessary for plants growing on –S soil/medium. We also demonstrate that induction of miR395 is controlled by SLIM1, a key transcription factor in the sulphur assimilation pathway. Unexpectedly, the mRNA level of a miR395 target gene, *SULTR2;1*, strongly increases during miR395 induction in root. We show that the spatial expression pattern of MIR395 transcripts in the vascular system does not appear to overlap with the expression pattern previously reported for SULTR2;1 mRNA. These results illustrate that negative temporal correlation between expression level of a miRNA and its target gene in a complex tissue cannot be a requirement for target gene validation.

Supra-optimal copper levels may disturb the regulation of uptake and metabolism of sulfate in plants

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Arable soils may be contaminated with copper as the consequence of unbalanced fertilization with manure and organic fertilizers or the use of copper-containing fungicides. Copper is an essential plant nutrient and it functions as redox-active transition metal in enzymes in many physiological processes, e.g. photosynthesis, respiration, oxidative stress response. However, at supra-optimal levels copper may become phytotoxic and the general symptoms are chlorosis and a stunted growth. For instance, exposure of Chinese cabbage (*Brassica pekinensis*) to supra-optimal Cu^{2+} levels (1-10 µM) resulted in leaf chlorosis, a loss of photosynthetic capacity and biomass production at ≥ 5 µM. The Cu content of the root increased with the Cu^{2+} concentration (up to 40-fold), though only a minor proportion (4 %) of it was transferred to the shoot. The nitrogen content of the root was hardly affected at suboptimal Cu^{2+} levels, whereas that in the shoot was decreased at ≥ 5 µM Cu^{2+}. The total sulfur of the shoot was increased at ≥ 2 µM Cu^{2+}, which could be attributed to an increase in sulfate content. Moreover, there was a strong increase in water-soluble non-protein thiol content in the root and to a lesser extent in the shoot at ≥ 1 µM, which could only partially be ascribed to a Cu-induced enhancement of the phytochelatins, thiol-rich compounds which may play a role in the homeostatic control of potential toxic metal ions in plants. The nitrate uptake by the root was substantially reduced at ≥ 5 µM Cu^{2+}, whereas that of sulfate was slightly enhanced or unaffected at 2 and 5 µM Cu^{2+}. The Cu-increased activity of the sulfate transporters was accompanied with an enhanced expression of sulfate transporters transcripts in both root and shoot. The up-regulation of the sulfate transporters could not be ascribed to a higher sulfur demand upon at supra-optimal Cu levels, but was more likely the consequence of the direct interference of Cu with the signal transduction pathway regulating the expression and activity of the sulfate transporters. The significance of gaseous H_2S as possible signal in the cross-talk between the sulfate reduction pathway in chloroplast/plastid and the transcription of sulfate transporters/sulfate-reducing enzymes will be discussed. The relevance of sulfur metabolism in the detoxification of copper will be evaluated.

Identification of plastidic transporter involved in the biosynthesis of Met-derived glucosinolates in *A.thaliana*

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The biosynthesis of aliphatic glucosinolates is highly compartmentalized process. It requires, for example, an import of Met-derived 2-oxo-acids into chloroplasts, side-chain elongation mediated by MAM1/3 (Textor et al., 2007) and the export of the resulting compounds into the cytosol for further conversion into glucosinolates. We could identify two isopropylmalate isomerases genes, *IPMI1* and *IPMI2,* the isopropylmalate dehydrogenase gene *IPMDH1* as well as BAT5 transporter as novel targets of HAG1/MYB28 transcription factor. The corresponding proteins were shown to be localised in plastids, signifying a role for these genes in chain-elongation reactions of 2-oxo-acids. The chloroplastidic transporter has further been studied in more detail and the *bat5* knock-out mutant has been shown to contain strongly reduced levels of aliphatic glucosinolates. *BAT5* mRNA was present in the mesophyll of leaves, vasculature, inflorescences, flowers and siliques, i.e. at sites where aliphatic glucosinolates are found. It is also shown that mechanical stimuli and MeJA induce expression of this transporter. The low glucosinolate chemotype of *bat5* mutant could be complemented by the overexpression of *BAT5* gene and in feeding experiments with 2-oxo-acids or side-chain elongated methionine. Altogether, our experiments suggest that BAT5 functions as a plastidic transporter for 2-oxo-acids, which are imported into the chloroplasts for chain elongation and exported into the cytosol for the conversion into Met-derived glucosinolates.

References:

Textor, S., de Kraker, J.W., Hause, B., Gershenzon, J., and Tokuhisa, J.G. (2007). MAM3 catalyzes the formation of all aliphatic glucosinolate chain lengths in arabidopsis. Plant Physiol. **144:** 60-71.

Influence of sulphur form and rate on growth of phytopathogenic fungi

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The targeted use of minerals offers a possibility to enhance resistance of plants against pathogens. Here, the direct toxicity of nutrients and indirect impairment by minerals needs to be distinguished from nutrient-mediated, resistance mechanisms. Soil-applied sulphate fertilisation proved to significantly reduce infection rate and severity of crops by fungal diseases under field conditions. Sulphur-Induced Resistance (SIR) is acknowledged in the science community as one constituent of the complex phenomenon of induced resistance (IR). Though relevant S compounds which are involved in SIR have been identified, it is yet not possible to trigger SIR after infection. Research focused on plant physiological aspects of SIR, while the response of the pathogen to varying S-containing metabolites and rates in terms of growth response hardly found attention. In the present investigation the influence of rated S applied as glutathione, methionine, cysteine, S^0, $MgSO_4$ and K_2SO_4 on colony growth of *Drechslera teres*, *Fusarium culmorum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* was determined. The results reveal that glutathione and cysteine reduced rate-dependently colony growth strongest by about 87% and 71%, respectively when the mycelium of the control covered the entire agar plate. In comparison, the highest S^0 rate (250 mg S/plate) reduced growth of *D. teres* and *S. sclerotiorum* by 63%, while it had only minor effects on *F. culmorum*.

Is the regulation of sulphate assimilation in *P. patens* like in higher plants?

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As an essential nutrient regulation of sulphate metabolism must be highly regulated. After uptake from soil and activation sulphate is reduced in order to synthesise in a last step cysteine. In higher plants the key step of this pathway is the reduction to sulphite through adenosine 5'-phosphosulphate reductase (APR). Whereas APR is feedback repressed by thiols and induced by reduced levels of glutathione (GSH). The aim of our work was to analyse if APR in *P. patens* reacts similarly.
Neither GSH nor buthionine sulfoximine (a well-known GSH biosynthesis inhibitor) treatment influenced mRNA levels of sulphate assimilation genes. As *P. patens* did not react as predicted further experiments were designed. O-acetylserine, cadmium and sulphur deficiency showed effects at APR transcript levels but not on enzyme activity. These results indicate that regulation of sulphate assimilation in *P. patens* functions in a different way. Because of contrary effects on transcript level and enzyme activity we assume that regulation of sulphur assimilation could be a post-transcriptional mechanism.
As a result, the moss pathway seems to be more robust to disturbances in sulphate supply than it is in higher plants.

Sulfur flux through the sulfate assimilation pathway is differently controlled by adenosine 5'-phosphosulfate reductase under stress and in transgenic poplar plants

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The key step of the sulfate assimilation is the reduction of activated sulfate, adenosine 5'-phosphosulfate (APS) to sulfite catalyzed by APS reductase (APR). We tested whether APR controls the flux through the sulfate assimilation pathway in poplar roots under a broad range of environmental conditions. Several treatments known to strongly affect APR activity by increasing the demand for reduced sulfur were selected to analyze [^{35}S] sulfur flux from external sulfate into glutathione (GSH) and proteins. (1) OAS treatment strongly pushed Cys synthesis and enhanced the sulfur flux through sulfate assimilation which was mainly controlled by APR activity (71% of control). (2) Cd that induces phytochelatine synthesis from GSH and (3) the herbicide Acetochlor which is detoxified via conjugation to GSH by glutathione S-transferase activity resulted in a loss of control by APR. The same loss of control by APR was observed when *Lemna minor* APR was overexpressed in poplar. However, the control of sulfur flux through sulfate assimilation mediated by APR was maintained in poplar roots when (i) the gene of the key step in glutathione formation, γ-glutamylcysteine synthetase (*γ-ECS*), was overexpressed either in the cytosol or in the plastids and, when (ii) the sulfite oxidase (*SO*) from *Arabidopsis thaliana* that catalyses the back reaction of APR, i.e. the reaction from sulfite to sulfate, was overexpressed. These results strongly support the view of a high significance of APR as a controlling step in sulfate assimilation, but show also the disturbance of control under certain environmental conditions.

Acclimation to stress and assessment of the 'metabolome' compartmentation in Arabidopsis

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Within their natural habitat plants are continuously subjected to single and combinations of abiotic stresses such as variable light intensity and nutrient starvation. Environmental stresses induce changes in the growth condition that alter or disrupt metabolic homeostasis. These modifications in growth require an adjustment of metabolic pathways including photosynthesis, aimed at achieving a new state of homeostasis, in a process that is usually referred to as acclimatization. To generate a better understanding of these cellular dynamics of plant acclimation in response to specific stresses plants were exposed to high light and sulfur depletion alone and in combination. Furthermore, compartmentation of metabolites as aspect of metabolic regulation was investigated for a fundamental understanding of plant metabolism for qualitative and quantitative description of the metabolome within different compartments of the cell. Knowledge of metabolic networks and their regulation can therefore be further developed based upon increased understanding of relative distributions of metabolites in these compartments as determined by experimental analyses.

A Proteomic Approach to Understanding DMSP Production in a Marine Diatom

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Marine phytoplankton play a key role in the global sulphur cycle through the production of dimethyl sulphoniopropionate [DMSP; $(CH_3)_2S^+CH_2\ CH_2COO^-$]; the direct precursor of the volatile, climate cooling, sulphur compound dimethyl sulphide [$(CH_3)_2S$; DMS]. Despite the importance of DMSP in the sulphur cycle little is known about the regulation of its production. The aim of this research is to use a proteomic approach to identify key proteins in the pathway of sulphate assimilation and DMSP production in the marine diatom *Thalassiosira pseudonana* (CCMP1335). Whilst diatoms commonly have low cellular DMSP, relative to phytoplankton in other major groups, it has been demonstrated that DMSP production is inducible in this species under a number of conditions including nutrient and carbon dioxide limitation. This and other data on high-DMSP content diatoms has attracted research interest in the global potential of DMSP production by this group. Importantly, the genome of this *T. pseudonana* has been sequenced. Here we have explored how changing light intensity, salinity and nitrogen availability affect intracellular DMSP concentration in this species. The proteomes of cells under these conditions will be compared via two-dimensional electrophoresis and mass spectrometry and key proteins in the pathway will be identified. The regulation of the activity and abundance of these proteins can then be investigated. This will increase our understanding of the biological processes that control the production of DMSP by phytoplankton in the marine environment and could contribute to future climate and biogeochemical models that incorporate production of DMS.

Characterization of early changes in gene expression of leaves of different developmental stages under sulphur deficiency conditions in poplars (*Populus tremula x P. alba*)

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Poplar is a continuously growing tree allowing the analysis of leaves of different developmental stages from one plant [4]. It is already shown for different vegetative plants that young expanding leaves are strong sink tissues for sulphate taken up by the roots [1, 2, 6]. With leaf maturation a transition from sink to source takes place whereas in mature leaves of poplar the most of the exported sulphate is transported basipetal to the roots [5]. It could be also shown for poplar that the activities of enzymes involved in sulphate assimilation differed between leaves of different developmental stages [5]. Under sulphur deficiency conditions the first deficiency symptoms are visible on young leaves [3].
Focussing on early reactions due to sulphur deficiency, we compared gene expression of different sulphate transporters and enzymes involved in sulphate reduction in young and mature leaves of poplars (*Populus tremula x P. alba*) grown in sand culture. During the whole experimental period of 21 days, no deficiency symptoms were visible on the plants. However, we found significantly changes in gene expression as well as in sulphate and thiol contents over the time. Comparing young, developing leaves and mature leaves, the regulation of gene expression according to sulphur deficiency was clearly different reflecting their unequal demand for sulphate.

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Microbial Arylsulfatases: Diversity and activity in the environment.

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In soil less than 5% of sulfur is inorganic and bio-available to plants. Sulfate for plant nutrition is therefore predominantly provided by microbial turnover of organically bound sulfur. Arylsulfatase is the best studied of the enzymes that mobilize this bound sulfur, and is found in both periplasmic and cytoplasmic forms, with different pH optima. The enzyme is encoded by the *atsA* gene, whose expression is controlled by the sulfur supply to the cell. Although sulfatase activity is routinely assayed in soils as a measure of soil health, there is a lack of detailed studies on arylsulfatase gene diversity in the soil. To address this, we undertook comparative analysis of the 248 putative bacterial arylsulfatase genes (*atsA*) identified on the NCBI database. These demonstrated high genetic diversity, but alignment enabled us to design degenerate primers and optimize PCR conditions for successful amplification of the *atsA* gene from a range of bacterial species, allowing phylogenetic analysis of *atsA* in soil. To validate these, we analysed bacterial isolates obtained from soil samples taken from 11 different sites in the Peak District National Park (UK) of different land usage and soil/bedrock type. These isolates were screened for arylsulfatase activity at the pH optima for the periplasmic and cytoplasmic forms. The influence of soil type and land usage on activity was investigated, and compared with the overall *atsA* gene diversity determined using the new primers.

Identifying "regulatory elements" of sulfur homeostasis

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We aimed to identify local and systemic regulatory elements of sulfur homeostasis in *Arabidopsis thaliana.* Sulfate starvation induces pleiotropic effects camouflaging the "real" –S response. Thus, the aim was to establish a system allowing to induce a –S response in sulfur supplied plants. Two approaches have been chosen to induce a –S response in a +S environment: a split root system being able to induce a –S response via systemic signals and an inducible overexpression of the enzyme serine acetyltransferase (SAT) elevating suddenly the cellular OAS levels. The results obtained in the split root experiment will be presented in detail. A split root system was established in hydroponic culture system and on agar plates to analyse metabolic and molecular response to -S conditions in partially starved roots. The current results revealed only a local response under –S conditions. Neither uptake capacities nor assimilation were enhanced on the +S side of roots when the other parts of the root system was sulfur depleted. However, a model in which the local availability of sulfate determines root growth and a sulfur rich shoot inhibits a costly degradation of sulfur compounds in roots which finally would weaken the root and thus the plant respectively, will be discussed.

Assembly of the cysteine synthase complex and the regulatory role of protein-protein interactions

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Macromolecular assemblies play critical roles in regulating cellular functions. The cysteine synthase complex (CSC), which is formed by association of serine O-acetyltransferase (SAT) and O-acetylserine sulfhydrylase (OASS), acts as a sensor and modulator of thiol metabolism by responding to changes in nutrient conditions. Biophysical examination of the soybean CSC by size-exclusion chromatography and sedimentation ultracentrifugation indicates that this assembly (complex M_r~330 kDa) consists of a single SAT trimer (trimer M_r~110 kDa) and three OASS dimers (dimer M_r~70 kDa). Analysis of the SAT-OASS interaction by isothermal titration calorimetry reveals negative cooperativity with three distinct binding events during CSC formation with K_d values of 0.3, 7.5, and 78 nM, respectively. The three binding events are also observed using surface plasmon resonance with comparable affinities. The stability of the CSC derives from rapid association and extremely slow dissociation of OASS with SAT, and requires the C-terminus of SAT for the interaction. Steady-state kinetic analysis shows that CSC formation enhances SAT activity and releases SAT from substrate inhibition and feedback inhibition by cysteine, the final product of the biosynthesis pathway. Cysteine inhibits SAT and the CSC with K_i values of 2 and 70 μM, respectively. These results suggest a new model for the architecture of this regulatory complex and additional control mechanisms for biochemically controlling plant cysteine biosynthesis. Based on previous work and our results, we suggest that OASS acts as an enzyme chaperone of SAT in the CSC.

Genetic dissection of demand-driven regulation of sulfate assimilation

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Sulfate assimilation is regulated by demand for reduced sulfur. Sulfate uptake and the activity of the key enzyme of sulfate reduction, adenosine 5'-phosphosulfate reductase (APR) are increased at high demand, e.g. during stress or depletion of reduced sulfur pools, e.g. by inhibition of glutathione synthesis with buthionine sulfoximine (BSO). On the other hand, they are inhibited when reduced S compounds are in excess. While the regulation of APR activity and mRNA accumulation is well described, little is known about molecular mechanisms of this regulation. We have utilised the induction of APR by BSO for a genetic screen with plant expressing luciferase under control of APR3 promoter to isolate mutants in the demand-driven regulation of APR. Ten *bis* (***B****SO* ***i****nsensitive*) mutants were isolated that were not able to induce luciferase and APR activity on BSO. We used transcript based cloning to identify the underlying genes. Two genes were isolated that have important roles in light regulation and perception. In addition, using the strong inhibition of root growth by BSO, we isolated several *bir* (***B****SO* ***i****nsensitive* ***r****oots*) mutants, that are affected in glutathione homeostasis. Cloning of the first bir mutant revealed an unexpected link between glutathione synthesis and function of mitochondrial respiratory chain.

Interaction of glutathione with flowering time

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The tripeptide glutathione (GSH) plays multiple functions in plant cells especially in connection with redox environment. During initial characterisation of mutants affected in GSH homeostasis we observed that a large portion of these mutants set flowers later than corresponding wild type. Indeed, detailed analysis of six mutants revealed that they initiated flowering 3 - 10 days later than Col-0 in long days and 9 - 16 days later in short days. In addition, these mutants possessed more rosette leaves at the time of bolting than Col-0. To investigate the link between GSH and flowering in more detail, we measured foliar GSH content in four genotypes differing in flowering time. Interestingly, the GSH levels in rosette leaves were high before flowering and dropped to about 30-40% after transition to reproductive phase. In addition, in three of these genotypes the ratio of oxidised GSH to total showed a sharp minimum directly after initiation of bolting. Mutants in GSH synthesis did not differ significantly from WT in flowering time in long days, but *rax1* (but not *cad2*) set flowers later at short days. Inhibition of GSH synthesis by BSO in the initial phase of vegetative growth resulted in induction of transcript for flowering time repressor FLC and consequently late flowering. Thus, glutathione is an important, although not a major factor in control of flowering time.

The significance of sulphur in marine biogeochemistry

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The ocean plays a key role in the global biogeochemical cycles that are vital for life on Earth. In the case of sulphur, nanomolar concentrations of dimethyl sulphide [$(CH_3)_2S$; DMS] can be readily measured in a wide range of marine aquatic environment. This volatile compound is the main natural vehicle for the transfer of sulphur between the sulphur-rich oceans and terrestrial ecosystems where sulphur can be in much shorter supply. There is also a connection between DMS, clouds and the Earth's climate because once in the atmosphere DMS rapidly oxidises to form acidic sulphate aerosol particles. These influence atmospheric chemistry and may also cool the Earth's climate directly or indirectly by acting as cloud-condensation nuclei. DMS derives from a zwitterionic precursor compound known as dimethylsulphonio-propionate (DMSP; $[CH_3)_2S^+CH_2$ $CH_2COO^-]$ which is found in a range of seaweeds and protists. DMSP appears to be a multifunctional compound: in phytoplankton cells it may function as a compatible solute, a cryoprotectant, an antioxidant, an overflow metabolite under conditions of unbalanced growth and a grazing deterrent or attractant. When cells leak, are compromised in some way or disintegrate due to autolysis, grazing or viral lysis some of the DMSP released is converted to DMS by the action of algal or bacterial enzymes. In this talk I will briefly review the topic from my biological oceanography perspective drawing upon evidence and examples from the literature and our research group.

The Role of 3'Region of Sulfate Transporter SULTR2;1 in Arabidopsis – Sulfur Limitation Response and Physiological Function

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Low-affinity sulfate transporter SULTR2;1 has been considered to function in sulfate translocation from roots to shoots because of its predominant localization in vascular tissues. The mRNA level of SULTR2;1 is highly up-regulated responding to sulfate starvation (-S) in roots but decreased in shoots. In this study, we identified the –S-responsive region of SULTR2;1 and found that –S-responsive expression of SULTR2;1 in roots requires the 3'non-transcribed region. In transgenic plants containing the SULTR2;1-5'region::GFP::SULTR2;1-3'region fusion construct, GFP accumulation was induced by –S not only in vascular tissues but in cortex. Physiological relevance of this 3'region was verified in plants containing T-DNA insertions in SULTR2;1-3'region (tKO). The –S response of SULTR2;1 was completely abolished in roots while unaffected in shoots by tKO. In addition, both the sulfate uptake and translocation were decreased in tKO under –S condition, suggesting that SULTR2;1 contributes to both processes through its induction driven by the 3'region in roots.

Genes of primary sulfate assimilation are part of the glucosinolate biosynthetic network in *Arabidopsis thaliana*

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Glucosinolates are sulfated, amino acid-derived metabolites involved in defense against herbivore and insect attack. The sulfate moiety is essential for the formation of biologically active products upon glucosinolate degradation. Its transfer from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a desulfated precursor is the final step in glucosinolate biosynthesis. PAPS is a product of sulfate assimilation, synthesized in a two step reaction catalyzed by ATP sulfurylase (ATPS) and adenosine 5'-phosphosulfate kinase (APK). Two groups of R2R3-MYB transcriptions factors are known to regulate aliphatic and indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. Using trans-activation assays we demonstrated that two forms of APK, *APK1* and *APK2*, are regulated by both classes of glucosinolate MYB transcription factor; whereas two ATPS genes, *ATPS1* and *ATPS3*, are differentially regulated by the two classes. Surprisingly, we found that all three adenosine 5'-phosphosulfate reductase (APR) genes of primary sulfate assimilation are also activated by the MYB transcription factors. Analysis of transgenic lines with modified expression levels of the glucosinolate MYB factors confirmed the MYB-dependent regulation of these genes. Enzyme activities and thiol levels were also affected in these transgenic lines. This study clearly shows that MYB factor control of glucosinolate biosynthesis extends to regulation of primary sulfate assimilation genes.

Impact of sulfur deficiency and hormone treatment on tobacco seedlings roots

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It is known that plant hormones play important role in plants response to sulfur (S) deficit affecting plants physiology and development. Trying to extend actual knowledge in this area we performed experiments with tobacco seedlings grown in the presence of hormones (JA, SA, ACC) in S-sufficient and S-deficient conditions. The seeds were germinated and cultivated on vertically placed Petri dishes for 3 weeks. We observed that hormone treatments reduced roots length in the conditions of sufficient S supply and inhibited roots elongation under S deficiency conditions. Roots architecture was changed by 1µM ACC, root system had some (usually 3 to 4) long roots despite of one main organ. Seedlings treated with 1µM JA were smallest and had pale leaves even on S-sufficient media. All three hormones greatly inhibited also leaves development in S deficiency conditions, while this effect was not observed in the presence of ACC and it was reduced in the presence of SA on S-sufficient media. We also noticed that, during S deficit, roots were changing color from almost white through yellow to almost brown. To confirm and further investigate the last observation the 2 week old seedlings were germinated on S-sufficient media and transferred to S-deficient conditions. After 3-5 days roots changed color into yellow and after 8-12 days they began to turn into pale brown. The pale brown color probably results from cell death because only yellow color could be inverted back into white after transfer to S-sufficient media. After examination of the yellow roots under the microscope we found yellow deposits inside the root cells.

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Adenosine 5' phosphosulfate kinase is essential for Arabidopsis viability

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In *Arabidopsis thaliana*, adenosine 5' phosphosulfate kinase (APK) provides activated sulfate for sulfation of secondary metabolites, including the glucosinolates. We have successfully isolated three of the four possible triple homozygous mutant combinations of this family. The APK1 isoform alone was sufficient to maintain WT levels of growth and development. All metabolites measured were also identical to WT under standard growth conditions. In contrast, plants retaining only the cytosolic APK3 or plastidial APK4 displayed a similar but slightly more severe growth phenotype than that described for *apk1 apk2* double mutant previously (Mugford *et al*., 2009, Plant Cell 21, 910-927), indicating that APK3 and -4 are capable of providing sufficient activated sulfate to sustain growth. The *apk1 apk2 apk3* and *apk1 apk3 apk4* mutants were phenotypically very similar to *apk1 apk2*, suggesting that APK3 and APK4 are functionally redundant, despite being located in different compartments. We were unable to isolate *apk1 apk3 apk4* mutants. Based on the absence of *APK2* transcript in the pollen as determined by promoter:GUS fusions, and the segregation ratios of progeny from doubly homozygous:heterozygous parents, we hypothesise that the *apk1 apk3 apk4* triple mutant combination is pollen lethal and that therefore APS kinase is essential for plant reproduction.

Differential regulation of serine acetyltransferase is involved in nickel hyperaccumulation in *Thlaspi goesingense*

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When growing in its native habitat *Thlaspi goesingense* (Brassicaceae) can hyperaccumulate up to 1.2% of its shoot dry weight as nickel (Ni), without showing symptoms of Ni toxicity. This is 100 – 1000 fold higher Ni accumulation than observed in other plants growing in the same habitat. We previously reported that the constitutively elevated concentration of the antioxidant glutathione (GSH) is involved in the ability of *T. goesingense* to tolerate Ni. Furthermore, the high concentrations of GSH in *T. goesingense* are also associated with constitutively elevated activity of serine acetyltransferase (SAT), a key enzyme in GSH biosynthesis. SAT is the first rate limiting enzyme in the biosynthesis of cysteine, a precursor of GSH, and this enzyme is involved in the biosynthesis of OAS from acetyl-CoA and serine. One of the features of SAT is that usually the cytosolic isoform is feedback-inhibited by $_{L}$-cysteine. To better understand the possible role of this allosteric regulation of SAT in GSH mediated Ni tolerance in *T. goesingense*, we characterized the enzymatic properties of the cytosolic (SAT-c), plastid (SAT-p) and mitochondrial (SAT-m) isoforms of SAT from *T. goesingense*. We demonstrate that all isoforms of SAT in *T. goesingense* are insensitive to inhibition by cysteine. This contrasts with the situation in the closely related Ni non-accumulator *Arabidopsis thaliana* (Brassicaceae), in which the cytosolic isoform of SAT is sensitive to cysteine. We have identified two important amino acid changes (Cys to Pro at 268th and Gly to Ala at 270th) in the C-terminal region of SAT-c from *T. goesingense*
that converts the enzyme from a cysteine sensitive to an insensitive isoform. We also observe that this change increases the binding affinity of the enzyme for it substrate serine, limiting the ability of cysteine to competitively inhibit the enzyme. Furthermore, we have established that the cysteine insensitive isoform of SAT confers elevated tolerance to Ni when expressed in *E. coli*, supporting a role for altered allosteric regulation of SAT by cysteine in Ni tolerance in *T. goesingense*.

Interaction between atmospheric and pedospheric sulfur nutrition and copper toxicity in chinese cabbage

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Sulfur is an essential element for plants and it has been known for several decades that foliarly absorbed sulfurous air pollutants (SO_2, H_2S) may be metabolized and may contribute to sulfur fertilization of plants. There is direct interaction between atmospheric and pedospheric sulfur utilization, for instance H_2S exposure may down-regulate the expression and activity of the sulfate transporters and APS reductase, the key regulating enzyme of the sulfate reduction pathway. Organic fertilizers may contain supra-optimal copper levels, which at strongly enhanced levels may become phytotoxic. It has been presumed that sulfur metabolites might play an essential role in copper homeostasis and detoxification in plants. In the present study the significance of the plant sulfur status in the detoxification of supra-optimal copper levels was evaluated. Chinese cabbage was exposed to supra-optimal levels of copper (5, 10 and 15 µM Cu^{2+}) in the root environment and the impact of sulfate-deprivation and H_2S exposure (0.2 1 l^{-1}) on growth, the activity and expression of the sulfate transporters and sulfur assimilation in the presence of supra-optimal Cu^{2+} levels was investigated.

The role of sulfonate desulfurizing *Polaromonas* sp. in sulfate limited soils

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The rhizosphere is a hot spot for bacterial activity where plant roots stimulate bacterial growth via exudation of carbon, but also deplete the rhizosphere of essential inorganic nutrients such as sulfate. In the absence of sulfate, some bacteria are able to utilize organo-sulfur sources such as sulfonates, a major component of soil sulfur. This study analyzed the diversity of desulfonating rhizobacterial communities and how they are affected by *i)* different long-term sulfur fertilization regimes in wheat rhizospheres *ii)* short-term sulfate starvation in grassland mesocosms *iii)* sulfate limitation in soil and pioneering plant rhizospheres in a natural chronosequence. Quantification of cultivable sulfonate desulfurizing bacteria showed two to eight fold higher numbers under sulfate limiting conditions when compared to a control experiment with added sulfate. Fertilization regimes, sulfate additions, plant host species and the soil chronosequence had a clear effect on the structure of desulfonating bacterial communities measured, using fingerprinting techniques with the key desulfonation gene *asfA*. Members of the genus *Polaromonas* containing *asfA* were found to dominate desulfonating communities under sulfate limiting conditions. The results suggest that members of the genus *Polaromonas* quickly become abundant when sulfate is limited, and that they play an important part in soil sulfur cycling, supporting the growth of crops, grasses and pioneering plants.

Serine Acetyltransferase Family in *Vitis vinifera*; the Peculiarity of VvSAT7

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Plants assimilate sulphur mostly through the formation of cysteine following sulphate absorption by roots and assimilatory reduction. *O*-acetylserine (*O*AS) and sulphide are substrates for cysteine synthesis, which is catalyzed by the enzyme *O*-acetylserine(thiol)lyase (OASTL). OAS production from serine is catalyzed by serine acetyltransferase (SAT). Both enzymes form the cysteine synthase complex, a protein multiplex which regulates cysteine synthesis and senses sulphate supply and demand. Partial sequences of *Vitis vinifera* genes *VvSAT* (EU275238) and *VvOASTL* (EU275237) were cloned using degenerate primers and deposited at GenBank. After the release of *Vitis vinifera* genome [1], [2] it was possible to identify sequences with high homology to *VvSAT* and *VvOASTL*. Seven SAT sequences could be identified that fell into five seemingly distinct groups. *V. vinifera* genome confirmed a high number of OASTL sequences as observed in other species and thirteen sequences were identified with high homology to *VvOASTL*. *Vitis vinifera* L. cv Touriga Nacional cell suspensions culture grown in liquid medium (+S, 1.5 mM sulphate) or without sulphate (-S) were used as model system to characterize and analyze *VvSAT* and *VvOASTL* gene expression. Up-regulation of *VvSAT7* after 5 days in –S conditions was observed and a mild up-regulation of *VvSAT6* after 7 days in –S conditions. Adding SO_4^{2-} to the –S cells leads to the repression of the up-regulation only in *VvSAT7*. mRNA levels of all *OASTL* sequences remained constant in all conditions. The possible localizations of VvSAT proteins in subcellular compartments will be discussed taking into account that SATs in *Arabidopsis thaliana* could be addressed to the cytosol, plastids and mitochondria.

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Regulation of leaf senescence in *old3* mutant of *Arabidopsis*

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Arabidopsis onset of leaf death (old) 3 mutant isolated from *Ler*-0 (Jing et al., 2002, *Plant J*) shows a temperature dependent leaf senescent phenotype with interaction between the *OLD3* gene and a natural variant gene, *ODD* (*old3* determination). *OLD3* encodes a mutated cytosolic *O*-acetylserine(thiol)lyase (OASTL) with single amino acid change, resulting in a complete lack of OASTL activity. Unlike the *old3* mutant, the senescent phenotype is not observed in T-DNA knockout mutant of cytosolic OASTL in Col-0 (*old3-T*) and *old3* mutant with *odd* gene from Col-0 (*old3odd*). This suggests that a differential regulation for senescence exists in *Arabidopsis* accessions. To investigate a role of *OLD3* in the regulation of senescence, we performed a temperature-shift experiment with *old3*, *old3-T*, *old3odd* and control plants. Plants were grown at 28°C for 16 days and transferred to either 20°C or kept at 28°C and grown for an additional 2-5 days. Plants including *old3* mutant grew normally at 28°C, but after the transfer to 20°C the *old3* mutant started senescence. We conducted OASTL activity assays and metabolite profiling. Based on the results, possible mechanisms will be discussed on how *OLD3* may be linked to leaf senescence.

Quantification of thiols at femtomolar level using laser-assisted microdissected fungal and plant material

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Complexity is a life's constitutive feature. Ignoring complexity by analysing bulk samples only average measurements of everything included in the sample can be performed. Plants/fungi are considered to contain about 40/28 (morphological) distinct cell types. The aim of this study was to establish cultivation, sampling and analysing methods for micro-metabolite profiling (often reported as *single cell sampling and analysis*) esp. for thiol peptides.
For any sampling method material integrity is of major concern. During (cryo)fixation, (cryo)microtomy, embedding and transfer techniques often biochemical alterations are noticed. Because of that, we developed on-slide cultivation techniques.
Cell walls complicate the use of single cell sampling techniques and we are still optimizing sampling parameters. Our microscopes are equiped either with manipulators/capillaries (invasive) or diode pumped passively Q-switched solid state UV-laser (355 nm, noninvasive).
Several studies have shown procedures for DNA extraction, RNA expression profiling and proteomic/metablomic profiling from 300-500, 1 000-5 000 and 20 000-30 000 microdissected cells, often of animal or human origin. As a result, we were able to determine transcripts of APR, APR-B and RuBisCO using 5-30 cells of *Physcomitrella patens*. CE separation of fluorescent (5-(iodoacetamido) fluorescein) labelled samples we were able to determine contents of femtomol thiols, which is equivalent to 300 fungal Spitzenzellen of *Heliscus lugdunensis*.

Significance of Sulfite Reductase in the Higher Plant *Arabidopsis thaliana*

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The regulation of assimilatory reduction of inorganic sulfate to sulfide in plants has long been controversially discussed. The significance of sulfite reductase (Sir) has been regarded as marginal for control of flux in this pathway, since transcript levels and extractable activity of Sir hardly respond to sulfur starvation. Two independent Arabidopsis T-DNA insertion lines (*sir1-1* and *sir1-2*) with an insertion in the promoter region of *Sir* were isolated. *sir1-2* plants had barely detectable *Sir* transcript levels and were seedling lethal, whereas *sir1-1* plants were viable, but severely affected in growth. *Sir* transcript levels in the leaves of *sir1-1* plants were down-regulated to about 60% of wild-type level and Sir protein and Sir enzymatic activity were reduced in the same manner. The uptake capacity for sulfate was strongly induced in roots of *sir1-1*, which explains in part the strong accumulation of oxidized sulfur in leaves of *sir1-1*. The significant differences between the leaves of *sir1-1* compared to wild-type plants for most of the sulfur-related metabolites and many genes of primary metabolism suggested strong perturbations in the entire metabolism and differences to the regulation of sulfate-deficiency-triggered gene expression in leaves. The 26- and 33-fold reduction of incorporation of ^{35}S label into cysteine and GSH in *sir1-1* showed that the activity of Sir generated a severe bottleneck in the assimilatory sulfate reduction pathway. The results rule out the existence of alternative pathways for sulfite reduction and show that its optimal activity is essential for the normal growth of Arabidopsis plants.

Functional characterization of ATP sulfurylase gene family in *Arabidopsis thaliana*

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In higher plants, sulfate is converted to adenosine 5'-phosphosulfate (APS) by the action of ATP sulfurylase (ATPS). APS can be a substrate of sulfur reduction, or can be phosphorylated to yield 3'-phosphoadenosine 5'-phosphosulfate, the sulfate donor for sulfation of a variety of compounds. In the *Arabidopsis* genome, there are four similar genes encoding ATP sulfurylase, *ATPS1*, *ATPS2*, *ATPS3* and *ATPS4*.
To analyze physiological roles of *ATPS* genes, we investigated the localization and function of these four ATPS isoforms. Tissue- and organelle-specific localization of ATPS was determined by expressing ATPS-GFP fusion protein under the control of *ATPS* promoter in *Arabidopsis* plants. ATPS1-GFP, ATPS2-GFP and ATPS3-GFP were expressed mainly in the vascular system of leaves and roots, whereas ATPS4-GFP was predominantly found in roots. Within the cell, ATPS1-GFP, ATPS3-GFP and ATPS4-GFP localized exclusively in plastid. By contrast, ATPS2-GFP localized both in plastid and cytosol, suggesting that the *ATPS2* gene encodes both the plastidic and the cytosolic forms of ATP sulfurylase. To investigate the function of ATPS in vivo, knockout mutants were isolated and analyzed for ATP sulfurylase activity. The analysis revealed that ATPS1 has the most dominant contribution for total APS production both in leaves and roots. To analyze which isoform is responsible for the APS synthesis in cytosol, we performed subcellular fractionation of leaves of wild-type and mutant plants. Only *atps2* mutant showed reduced activity of ATP sulfurylase in cytosol fraction, indicating that dual-targeting ATPS2 substantially contributes to supply the cytosol with APS.

Effects of sulfur treatment on glutathione contents and disease development in TMV-infected tobacco plants

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Sulfur is an essential macro-element for plant life and fulfills various biological functions in plant metabolism and defense. The current decline in atmospheric sulphur deposition has contributed towards a decreased capability of plants to fight biotic stress. Especially during fungal infections a sufficient supply of sulphur can lead to sulphur induced resistance (SIR). The exact mechanisms behind SIR remain unclear and also if SIR is a commonly found phenomenon also in other types of plant pathogen interactions. One of the most important sulphur containing antioxidants is the tripeptide glutathione. Elevated levels of glutathione are thought to be involved in the development of disease resistance by detoxifying reactive oxygen species (ROS) and the activation of defence genes.
The aims of the present study were to investigate if different degrees of sulphur supply can be correlated with changes in disease development in tobacco plants infected with tobacco mosaic virus (TMV) and if changes in glutathione metabolism on the subcellular level correlate with possible changes in symptom development.
Plants grown on sulphur depleted media developed symptoms of TMV disease earlier and stronger and also contained higher TMV contents than plants grown on media with sufficient amounts of sulfur. Glutathione contents were much stronger decreased in TMV-infected plants grown on sulfur depleted media than in the same plants grown on media with sufficient sulfur. Similar results were found for cysteine contents indicating that glutathione metabolism is strongly affected by TMV-infection and sulfur depletion.
The implications of these results will be discussed with respect to other parameters such as sulphur content in soil and leaves and changes in symptom development and virus contents.

Effect of sulfur nutrition on glutathione content in sugar beet plants in relation with aphids infestation

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The effects of sulfur (S) nutrition on plant growth and biosynthesis of glutathione (GSH) in leaves in relation with their infestation with aphis were studied in young sugar beet (*Beta vulgaris*) plants grown at constant cultivation conditions on soil fertilized with S and nitrogen (N). The nutrients in the form sodium sulfate of and ammonium nitrate were applied at different ratio into the soil before sowing. Plants were treated with black bean aphids (*Aphis fabae*) at age of 23 d and yielded the 7th d after the treatment. Both nutrients applied separately stimulated plant growth but their effects were markedly increased when supplied together. Fertilization with single N significantly increased infestation of plants with aphids. Addition of S to N decreased the stimulatory effect of N on aphids reproduction. Sulfur supplement highly enhanced content of GSH in leaves of all ages. Only in the oldest leaves on combined variants also the feeding aphids caused significant increase of GSH content. The role of GSH in resistance of plants against sucking insects will be discussed.

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Effect of nitrogen and sulfur fertilisation on growth of plants, health, yield, lycopene content and colour of tomato fruits

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Tomatoes are important source of lycopene in human nutrition. The effects of nitrogen (N) and different sulfur (S) fertilisers (ammonium, sodium, potassium and calcium sulfates) on plant growth, chlorophyll content, health, yield and quality of tomato fruits in two dwarf cultivars Proton and Šejk were investigated. Single N, applied as ammonium nitrate, stimulated growth of plants and significantly increased yield of fruits, but did not change content of lycopene as well as color parameters (a*, b* and L*) and decreased significantly S content in fruits. All S fertilizers significantly increased S and lycopene content in fruits (up to 39% in cv. Šejk and 92 % in cv. Proton) and positively influenced color of tomato puree, namely parametr a*. The earlier cv. Šejk responded better to S supply than var. Proton, which showed a negative yield effect esp. on variants where higher S doses were applied. However cv. Šejk was more susceptible to "blossom end rot" disease. Sodium sulfate undesirably very significantly enhanced Na content of fruits in both cultivars.

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UP15 protein and its potential role in coordination of S and N metabolism in tobacco

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The *UP15* gene from tobacco was identified in our laboratory as a gene induced by sulfur deficit. The –S response of *UP15* was confirmed by quantitative RT-PCR (qRT-PCR), however, the gene appeared to be induced also by nitrogen deprivation, even stronger than by sulfur deficiency. The *UP15* gene encodes a small (168 aa) Gly-rich protein of unknown function. Nuclear localization signal (NLS) is present near the C-terminal part of this protein, however, *in silico* analysis pointed out that localization in chloroplasts is also possible. Interestingly, transiently expressed in *N. benthamiana* leaves the UP15:GFP protein was found in both compartments, nucleus and in chloroplasts. Several possible homologues with very low similarity to UP15 exist in *A. thaliana.* At least one is annotated as a transcriptional factor with theoretically dual localization (nucleus and chloroplasts) in plant cell. To determine function of UP15 and to identify protein partners that interact with this protein *in vivo* under S-deficit yeast-2-hybrid (Y2H) system was applied. The most frequently obtained clones from Y2H contained fragments of a gene encoding ATase. This protein localizes in stroma of chloroplast and is responsible for the first step of purines biosynthesis and for metabolic change of glutamine into a glutamate.
Our results suggest that UP15 may play a role in adaptation of plant metabolism to the imbalanced nitrogen-sulfur supply due to reduced availability of either sulfur or nitrogen source. However, the precise role of UP15 in this regulatory network is still unknown.

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The phenotypes of Arabidopsis mutants for the group 3 sulphate transporters point diverse roles within developing seed

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Sulphur is an essential macronutrient needed for the synthesis of many cellular components. In particular, sulphate reduction leads to the synthesis of stress response-related compounds (e.g. glutathione, glucosinolates) and of sulphur amino acids, which are important determinants of seed nutritional value. The delivery of sulphate into plant tissues and cell compartments requires specific sulphate transporters. In the present study, the function of low-affinity plasma membrane transporters of group 3 was investigated in Arabidopsis. First, expression patterns of these five genes revealed an expression at specific stages of seed development. To provide information about their contribution to the establishment of seed composition, T-DNA mutants were characterized for each gene of this group. This study demonstrated a striking increase of sulphate content in the *3;2, 3;3, 3;4* and *3;5* mutants, but an unchanged total seed sulphur content, suggesting an alteration of sulphate exchange and reduction within developing seed tissues. Some mutants were strongly affected in seed storage protein synthesis and processing (e.g. the *3;5* mutant), whereas others are suggested to counteract the negative influence of the mutation through compensatory mechanisms (e.g. the *3;4* mutant). These changes were accompanied by a reduced primary root elongation, which could reflect an inability of the seedling to mobilise and/or transport the seed reserves.

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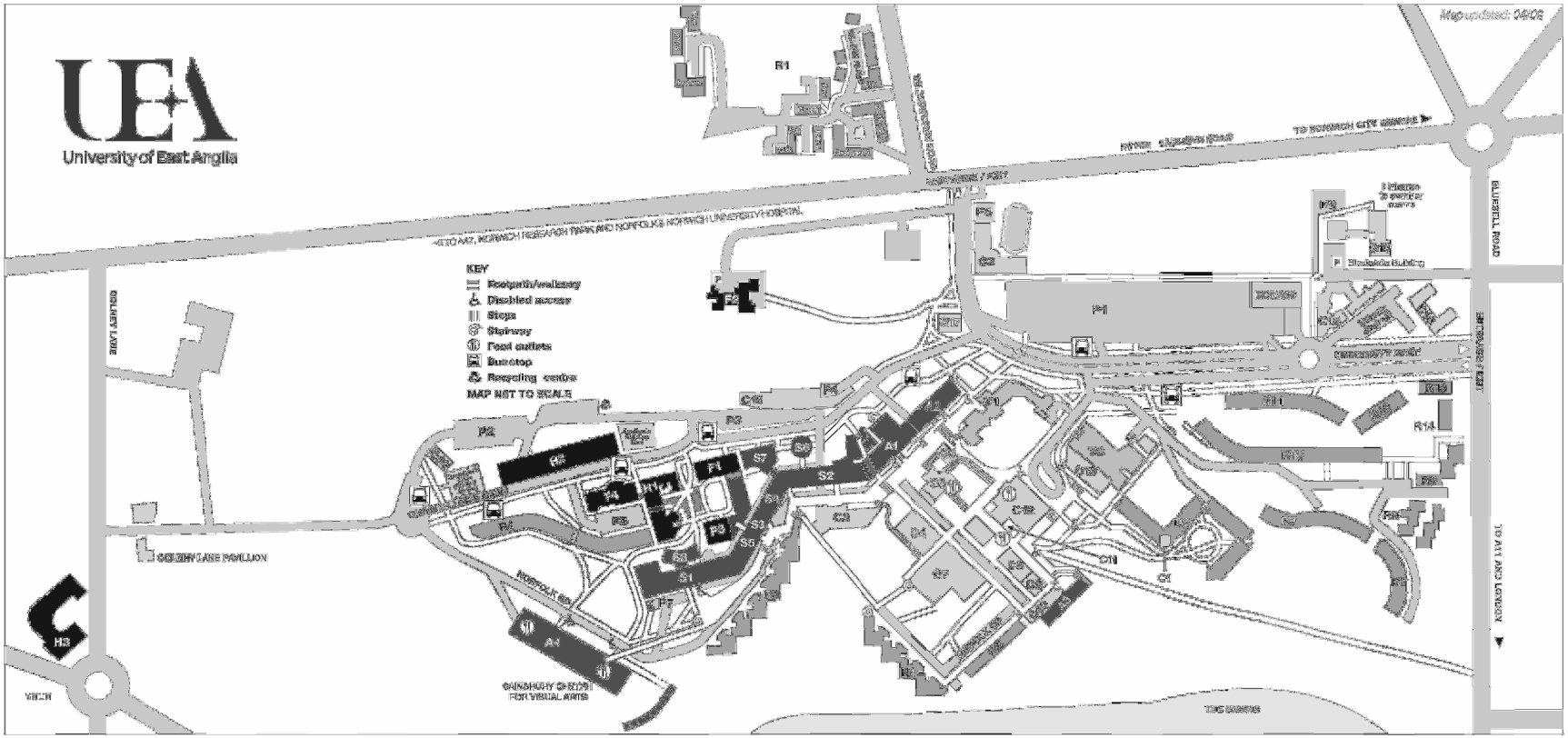

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- P7 SCVA Car Park (SCVA visitors only)
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Vice-Chancellor's Office and Administration (V)

- V1 Registry Academic Division, Marketing & Communications, Council House, Finance, Human Resources, Print Services, Registrar & Secretary's Office, Research, Enterprise & Engagement, Residences & Services, Vice-Chancellor's Office, Visitors' Reception
- V2 Congregation Hall: Recruitment, International & Outreach Office

Campus Services (C)

- C1 Conference Office
- C2 Sportspark
- C3 IT & Computing Service (ITCS)
- C4 Lecture Theatres
- C5 Restaurants
- C6 Chaplaincy
- C7 Library, Audio Visual Services
- C8 Dean of Students' Office (DOS)
- C9 Counselling Service
- C10 Careers Centre
- C11 The Street: Waterstone's Bookshop, NatWest Bank, Barclays Bank, Post Office, Paper Shop, Accommodation Office, Union Food Outlet, Launderette, Dixons Villa Coffee Shop
- C12 Union House: Travel Shop, The Hive/LCR, EmployAbility Job Shop, Student Union Advice Centre, Union Bars
- C13 UEA Shop
- C14 Prayer Hall
- C15 INTO
- C16 University Medical Centre: University Dentist, UEA Nursing, Launderette, Pharmacy
- C17 Security Lodge
- C18 Estates & Buildings Division
- C19 Blackdale Building: Faculty of Social Sciences, CUE East, Teaching Rooms