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P7–SULPHUR METABOLISM IN PLANTS – INTEGRATING COMPLEXITY

Organised by M.J. Hawkesford for the Plant Metabolism Group and sponsored by the Journal of Experimental Biology

P7.1 Whole plant regulation of sulfur uptake and assimilation in relation to both the need for growth and the potential sink capacity for secondary sulfur compounds

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In general plants utilize sulfate taken up by the roots as sulfur source for growth and prior to its assimilation sulfate needs to be reduced to sulfide before it is metabolized into organic sulfur compounds. Under normal conditions the rate of uptake and assimilation of sulfur will be in tune with the plant's sulfur need for growth. The rate may strongly vary between species and it is likely to fluctuate during the plants' life cycle (vegetative growth period, seed production). The tuning/regulation of the rate of sulfate uptake and assimilation in relation to the sulfur need for growth and adaptation to changes in environmental conditions is rather complex. The current information on the shoot/root co-ordination of the uptake of sulfate *versus* its assimilation and the signal transduction pathways therein involved is rather ambiguous. There may be a fast response regulation to sudden changes in the environment, for instance sulfur supply, via metabolite activation/deactivation and expression/de-repression of genes encoding the sulfate transporters and the enzymes involved in sulfur assimilation. In addition, changes in tuning/regulation may occur via altered growth patterns (*viz.* changes root development and/or shoot/root ratio). It is still largely unclear to what extent sulfate itself and/or other metabolic products of the sulfur assimilation are directly involved in sensing or act as regulatory signals.

Atmospheric H₂S impact studies are helpful as tool to get insight into the regulation of sulfate uptake and sulfur assimilation and the dissection of the signal transduction pathways involved. In addition to pedospheric sulfate, plants are also able to utilize foliarly absorbed H₂S as sulfur source and in some plant species there appears to be a good co-ordination between roots and

shoots in the tuning of the rates of sulfur uptake and its reduction/assimilation. This will be illustrated by recent data on the interaction between atmospheric H₂S and pedospheric sulfate nutrition in *Brassica* and *Allium*, species which strongly differ in sulfur need for growth as well as sink capacity for secondary (reduced) sulfur compounds.

P7.2 Specialised functions of sulphate transporter isoforms for the movement of sulphate within the plant

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Sulphate is the main form in which sulphur is taken up by plants, and is the predominant form for intracellular, cell to cell and long distance sulphur movement within the plant. The uptake of sulphate by the root is proton coupled. In recent years a gene family of plant sulphate transporters, which may be further subdivided into 4 groups, has been identified with examples from many different plant species. It appears that each group represents sulphate transporters with distinct kinetic properties, patterns of expression and or cell/tissue specificity that enable specific roles in the uptake and distribution of sulphate. High-affinity sulphate uptake, low affinity vascular transport as well as vacuolar efflux is controlled by the nutritional status of the plant, most notably with an apparent increase in capacity for sulphate uptake/ efflux during a period of sulphate deprivation. In addition, non-nutritionally regulated sulphate transporters are involved in movement within the plant. Within the Groups, the individual sulphate transporters may be further subdivided by differences in expression patterns on temporal, cellular or tissue basis, regulated by the nutritional status of the individual tissue, allowing a balanced movement of sulphate between sink and source tissues. A review on the specialised functions of the sulphate transporter isoforms will be presented.

P7.3 Structure-function approaches for sulfate transporters

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The function of a protein is strongly related to its primary sequence which is one of the determinants of its 3D structure. Interestingly, bioinformatics analysis of the primary sequences of proteins which belong to a multigenic family reveal the presence of conserved regions. In addition, amino-acid sequence comparisons of membrane proteins whose membrane transport function has been identified or postulated, can contribute to the revelation of consensual motifs. These conserved sequences can be assumed to be essential for the protein function to proceed. To date in the field of plant membrane transporter study, the role of these conserved sequences has not yet been clearly understood. Studying protein structure-function relations will contribute to a better understanding of the role of such sequences in membrane transporter regulation. Protein structures can be modified by site-directed mutagenesis and the function of the mutated transporters assayed by using appropriate heterologous expression systems. Our contribution shall contribute to a better understanding of the functional regulation of plant sulfate transport by cross-comparing the protein sequences of several H⁺-sulfate transporters as well as Na⁺-dependent and Na⁺-independent sulfate transporters isolated from organisms of different kingdoms.

P7.4 APS reductase – the key enzyme of plant sulfate assimilation

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The sulfate assimilation pathway provides reduced sulfur for the synthesis of amino acids cysteine and methionine, essential building blocks of proteins, and further sources of reduced sulfur for coenzymes and various secondary compounds. Flux control analysis revealed the enzyme adenosine 5'phosphosulfate reductase (APR) to possess the highest control over the pathway. APR was only recently identified to be the enzyme used by plants to reduce activated sulfate to sulfite. An extended biochemical analysis of APR identified an iron-sulfur center as a cofactor and revealed that the first reaction product is a free sulfite. In the past, two alternative pathways, via 'bound sulfite' or via PAPS reductase, an enzyme alternative to APR found in fungi and some bacteria, were proposed to exist in plants. An attempt to prove that plants use exclusively APR for sulfate assimilation, using targeted knockouts in the

moss *Physcomitrella patens*, however, resulted in cloning of PAPS reductase from this plant. The phylogenetic analysis of APR together with biochemical analysis of bacterial APR homologues resulted in discovery of a new type of bacterial assimilatory APR which also possesses an iron-sulfur cluster. We conclude that reduction of APS is much more common than previously expected and that the ability to use APS for reduction is dependent on the presence of the iron-sulfur cluster. However, our knowledge of the evolution of sulfate assimilation and the origin of plant APR remains fragmentary.

P7.5 The biochemistry of APS reductase

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Abstract not supplied

P7.6 Overproduction of SAT and/or OAS-TL in transgenic plants—a survey of effects

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Two last steps of cysteine biosynthesis are catalyzed by the two enzymes capable of forming the complex, serine acetyltransferase (SAT) and cysteine synthase (CS), called also O-acetyl-serine (thiol) lyase (OAS-TL). SAT is responsible for the production of O-acetyl-serine (OAS) from serine and acetyl-coenzyme A, while CS catalyzes formation of cysteine from OAS and hydrogen sulfide. Several distinct nuclear genes for SAT and CS enzymes exist in plants. Products of these genes are targeted into at least three cellular compartments: cytosol, chloroplasts and mitochondria. The SAT and CS enzymes are strongly evolutionary conserved, both structurally and functionally. Therefore, isoenzymes from various cellular compartments can be substituted not only by their plant counterparts from the other cellular compartments but also by their bacterial homologues. During the last decade transgenic plants overproducing SAT, CS or both enzymes simultaneously were obtained independently by several research groups. These manipulations led not only to the elevated levels of the respective products, namely OAS and cysteine but also to the increased amount of glutathione and changes in the levels of other metabolites and enzymatic activities. In several cases the transgenic plants were additionally shown to be less susceptible to the applied abiotic stresses. In this review we discuss and summarize all published and some of our unpublished results related to heterologous overproduction of SAT and CS in transgenic plants.

P7.7 Functional analysis of sulfurtransferase in higher plants

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Sulfurtransferases/rhodanases (STs) comprise a group of enzymes widely distributed in Archaea, Bacteria, and Eucarya that catalyse the transfer of a sulfur atom from suitable sulfur donors to nucleophilic sulfur acceptors. The best characterized ST is bovine rhodanese which catalyses *in vitro* the transfer of sulfane sulfur from thiosulfate to cyanide, leading to the formation of sulfite and thiocyanate. However, *in vivo* neither substrates nor sulfur acceptors could be clearly identified in any of the organisms investigated. Therefore, despite the presence of ST/rhodanese activities in many living organisms, the physiological role of the members of this multi protein family has not been established unambiguously. In *Arabidopsis* 18 ST-like proteins containing typical rhodanese signatures or domains have been identified by using different strategies to mine the databases. A couple of very similar *Arabidopsis* STs consisting of two domains (N- and C-terminal) of nearly identical size, similar to the structure of the bovine rhodanese, and several single domain ST/rhodanese proteins were isolated and characterized: The recombinant STs possess different *in vitro* enzymatic activities. The ST proteins are localized in different cellular compartments as demonstrated by transient expression of fusion constructs with the green fluorescent protein. The STs are differentially expressed in dependency on the light/dark rhythm, the developmental stage, and the nutritional status. Recent results on the functional *in vivo* analysis of members of this diverse protein family will be presented.

P7.8 Analysis of the promoter elements required for the regulation of cytosolic OASTL in Arabidopsis

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The expression of the *Atcys-3A* gene coding for one of the cytosolic isoforms of *O*-acetylserine(thiol) lyase from *A. thaliana* is highly regulated by abiotic stress, such as the presence of heavy metal and salt stress. The hormone ABA also mediates the regulation of *Atcys-3A* gene expression by salt. Besides, this gene shows a tissue-specific expression, with the highest expression in trichomes, which is observed very early in trichome cell development. To further investigate the regulation of the *Atcys-3A* gene we are characterizing its promoter region by fusion to a GFP reporter gene. An 1809 nt fragment containing the promoter, the 5'untranslated region, the first intron and two exon fragments is able to mimic the

expression pattern of the *Atcys-3A* gene when fused to GFP. We have made series of 5' and 3' deletion constructs to characterize the minimal cis-elements responsible of the abiotic stress regulation and the trichome-specific expression. For each construct, we generate different transgenic plants and visualize the GFP fluorescence by Confocal Laser Scanning Microscopy. In addition, we quantify the amount of the GFP protein in the transgenic plants by enzyme-linked immunosorbent assay (ELISA). Initial results suggest that the first intron contained in the 5'-untranslated region may act as enhancer-like sequence for the trichome-specific expression.

P7.9 Enhanced GSH synthesis as a means of regulating cellular redox state during stress

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Glutathione has many functions in plant cells, including redox signalling and regulation of gene expression. The glutathione redox couple is also considered to be a key player in homeostatic adjustment of the cellular redox potential in plant and in animal cells. We have explored the role of glutathione in signalling leading to changes in gene expression and have estimated the influence of the glutathione redox couple over the cellular redox potential. We will show that the free calcium signature of tobacco leaf discs is modified in a specific manner following exposure to either reduced glutathione (GSH) or glutathione disulphide (GSSG), and that gene expression is modified. To investigate the intercellular control of glutathione synthesis and its influence on leaf redox state in response to short-term chilling, maize leaves were subjected to two days growth at low growth temperatures (chill). This treatment had no effect on leaf phenotype, whereas return to optimal temperatures (recovery) caused extensive leaf bleaching. A 2-fold increase in both leaf cysteine and γ -glutamylcysteine occurred during the chill but leaf total glutathione significantly increased only in the recovery period, when the GSH/GSSG ratio decreased 3-fold. The relative roles of GSH and GSSG in phenomena that underpin leaf chlorosis will be discussed, particularly the contribution of the enhanced GSH accumulation to cellular redox balance.

P7.10 Control of assimilation and glutathione synthesis: interaction with N and C metabolism

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Sulfate assimilation is an essential pathway being a source of reduced sulfur for various cellular processes. Many reports showed that sulfate assimilation is well coordinated with the assimilation of nitrate and carbon. It was long known that during nitrate deficiency sulfate assimilation is reduced and that the capacity to reduce nitrate is diminished in plants starved for sulfate. Only recently, however, it was shown that adenosine 5'phosphosulfate reductase (APR), the key enzyme of sulfate assimilation, is regulated by carbohydrates. In plants treated with sucrose or glucose APR was induced whereas the activity was strongly reduced in plants grown in a CO₂ free air. The molecular mechanisms for the coordination of S, N, and C assimilation are not known. O-acetylserine, a precursor of cysteine, was proposed to be the signal regulating sulfate assimilation, but most probably is not the outgoing signal to N and C metabolism. cDNA arrays revealed induction of genes involved in auxin synthesis upon S-starvation pointing to a possible role of phytohormones. Clearly, despite significant progress in understanding the regulation of sulfate assimilation and its coordination with N and C metabolism was achieved, our knowledge is far from being sufficient.

P7.11 Regulation and compartmentation of glutathione biosynthetic enzymes

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Higher plants respond to biotic and abiotic stress factors with an increase in glutathione (GSH) content. While cDNAs for the enzymes catalyzing GSH biosynthesis, GSH1 (γ -glutamylcysteine synthetase) and GSH2 (glutathione synthetase), have been cloned for many plant species, the complexities of regulation and subcellular compartmentation are only partially understood. Also, as GSH biosynthesis appears to be limited to the plastids, other cellular compartments requiring GSH for maintenance of the redox status have to import GSH, emphasizing a crucial role for intracellular GSH transport. Here we summarize recent research on the compartmentation of the GSH1 enzyme in *A. thaliana* and *B. juncea*. The analysis of multiple *GSH1* transcripts, *in vivo* targeting studies with GSH1::GFP(RFP) fusions, and the immunolocalization of GSH1 protein all confirmed an exclusive plastidic localization of the GSH1 enzyme. Conversely, a similar analysis for GSH2 indicated the presence of transcripts encoding plastidic and cytosolic proteins. As GSH is not rapidly exchanged between

plastids and cytosol, the dipeptide γ -glutamylcysteine is a likely candidate for plastid exit. The promoter of the *AtGSH1* gene is strongly up-regulated in response to Cd and jasmonic acid exposure, confirming that the increase of GSH1 protein observed under these treatments results, at least in part, from a transcriptional up-regulation of the *GSH1* gene. Interestingly, the promoter of the *AtOPT3* gene, a homolog of a GSH transporter from *B. juncea*, *BjGT1*, (Bogs *et al.*, 2003), is also up-regulated by Cd, indicating that heavy metal exposure affects both GSH biosynthesis and transport.

P7.12 Regulation of gene expression and transport functions: S responsive regions of sulfate transporter gene promoters and regulatory factors

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Plants require the function of plasmamembrane-bound transport machineries for the initial uptake of inorganic sulfate. During sulfur limitation, plants are able to activate the expression of sulfate transporters that facilitate the uptake of sulfate in roots. In Arabidopsis, *SULTR1;1* and *SULTR1;2*, are suggested to be the essential components of the sulfate uptake system. The physiological importance of *SULTR1;1* and *SULTR1;2* is supported by their characteristics ideal to cope with sulfur deficiency: They were (i) functional high-affinity sulfate transporters; (ii) induced by sulfur limitation at the mRNA levels; and (iii) predominantly localized in the root hairs, epidermis and cortex. The high-affinity sulfate transporters were primarily regulated by the demands of sulfur nutrition. *SULTR1;1* was regulated by sulfur limitation at the level of mRNA transcription, and was under the control of protein phosphatase. We identified a S responsive region in the -3 kb promoter of *SULTR1;1*. By contrast, the S responsive region of *SULTR1;2* was delimited within the -0.5 kb promoter. The analysis of promoter-GFP plants indicated that *SULTR1;1* and *SULTR1;2* are negatively regulated by a plant hormone, cytokinin. The *cre1-1* cytokinin receptor mutant showed marked reduction of cytokinin sensitivity for the repression of sulfate uptake, suggesting that cytokinin-derived signaling circuitry is involved in the regulation of *SULTR1;1* and *SULTR1;2* in Arabidopsis roots.

P7.13 Transcriptome and metabolome analysis of plant sulfur metabolism

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Nutrient ion assimilation needs to be tightly controlled at the biochemical level and to be interlinked with the network of plant metabolism. As availability and distribution of nutrient ions is neither in space nor in time predictable for immobile plants we have to assume flexible adaptation mechanisms. Sulfur is one of the major plant nutrients. We challenged *Arabidopsis thaliana* with varying concentrations of sulfate at their rhizosphere. The primary responses after sensing alterations in nutrient availability are to be expected at the level of gene regulation which led us to compare the transcriptome of plants under sulfur depleted in comparison to sulfur sufficient conditions. The ultimate effect of the physiological response processes have to be expected at the metabolite level. Therefore we performed a metabolite profiling of the respective plant material. Hundreds of changes at the transcript level and dozens of detected changes at the metabolite level can be mirrored on known pathways to visualize the network response and draw conclusions on interlinkage of respective pathways. Yet, to further deduce important elements in the systems response we applied bioinformatic tools such as clustering and mutual information content analysis on a fused transcript – metabolite dataset. We propose that such an analysis helps to identify relevant key elements in the system. The analysis of these elements will put forth new knowledge on the regulation of sulfur metabolism.

P7.14 Transcriptome and metabolome analyses reveal a whole adaptive process of plant to sulfur deficiency

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Plants have mechanisms to adapt to sulfur (S) deficiency and optimize metabolic balance according to nutritional status given to plants. To elucidate a whole adaptive response, we analyzed transcriptome and metabolome of *Arabidopsis* under $-S$ and related nutritional stresses. To elucidate long-term responses, *Arabidopsis* was grown under continuous $-S$, $-N$ (nitrogen) or $-SN$ conditions. On the other hand, to clarify early responses and adapting process to $-S$, plants grown under $+S$ were shifted to $-S$ and harvested at 3, 6, 12,

24, 48 and 168 hour-after-transfer. Plants grown under $+S$ were also transferred to *O*-acetylserine (OAS)-supplemented medium. In both experiments, plants were grown apparently normal. Transcriptome and non-targeted metabolome of leaves and roots were analyzed using DNA array and Fourier-transform ion cyclotron MS, respectively. Targeted metabolic profiling was also conducted using HPLC and capillary electrophoresis.

Global changes in transcriptome and metabolome were clearly shown by principal component analysis. It revealed the difference of responses between in leaves and in roots, and also time-dependent changes in response. OAS-treatment mimicked $-S$ in global changes in transcriptome and metabolome, suggesting that OAS is one of the positive regulators of not only gene expression in sulfate uptake and assimilation pathway, but also global metabolism under $-S$. On the other hand, self-organizing map analyses were performed to classify genes and metabolites according to their expression and accumulation patterns, respectively. Specific responses to $-S$ and general responses to nutritional stresses were suggested. For example, glucosinolate metabolism was shown to be regulated in time-dependent and treatment-specific manner.

P7.15 Current understanding of the regulation of methionine biosynthesis in plants

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Major crops, such as cereals and legumes, are low in cysteine and methionine and an attempt to manipulate the biosynthetic pathway is a major interest of molecular plant breeding. The accumulation of these amino acids in crops would increase the nutritional quality. It can be assumed that Met synthesis, accumulation and consumption are under tight regulatory control. It is therefore of importance to understand the physiological, biochemical, and molecular mechanisms that contribute to their transport, synthesis and accumulation in plants. This knowledge can be used to develop strategies allowing a manipulation of crop plants to eventually improve their nutritional quality.

This paper serves to highlight some recent findings linked to the metabolism of methionine in plants and its regulatory influence on the aspartate pathway and its implication in plant growth. In recent years, several key steps have been identified at the molecular level, enabling us to initiate transgenic approaches to engineer the methionine content of plants. Recent studies suggest that Met synthesis in plants has to be controlled at the level of competition between CgS and TS for their common substrate OPHS. Other studies suggest that the sulfate

reduction pathway may be limiting the sulfur amino-acid biosynthesis. In this paper, a summary of our current understanding of the regulatory network with the focus on efforts to understand and manipulate the carbon flux into Met is given.

P7.16 Understanding and manipulating sulfur accumulation in seeds

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Evidence supporting the hypothesis that reproductive sink organs are active in sulfur assimilation is summarised. Developing seeds of chickpea (*Cicer arietinum* L) and of wheat (*Triticum aestivum* L) were able to assimilate S supplied *in vitro* as sulfate, and contained quantitatively significant activities of the cysteine biosynthetic enzymes, serine acetyl transferase and O-acetylserine (thiol) lyase. Developing embryos of chickpea accumulated sulfate *in vitro* with kinetics consistent with active, low affinity sulfate transport. Furthermore, RT-PCR analysis identified putative sulfate transporter transcripts present in developing seeds of both dicots and monocots. In order to increase the storage of the nutritionally essential sulfur amino acids in seed protein, grain legumes and cereals were transformed with genes encoding a protein sink for cysteine and methionine, resulting in different effects on the composition of the different types of seeds. In grain legumes, accumulation of the methionine-and cysteine-rich sunflower seed albumin was associated with increases in total seed sulfur amino acids, indicating an increase in sulfur assimilation in the developing seeds in response to the added sink for organic sulfur. The transgenic seeds also displayed alterations in the relative abundance of endogenous storage proteins that resembled the effects of sulfur nutritional stress. Analysis of transgenic chickpeas grown in conditions of controlled mineral nutrition suggested that seed composition was modulated by free methionine and OAS in response to the changes in both sulfur demand and sulfur and nitrogen nutrition. Similar signalling pathways appeared to operate in transgenic cereals expressing the sunflower albumin.

P7.17 Biosynthesis of the flavour precursors of onion and garlic

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Onion (*Allium cepa*), garlic (*A. sativum*) and other Alliums are important because of the culinary value of their flavours and odours. These are characteristic of each species and are created by chemical transformation of a series of volatile sulphur compounds generated by cleav-

age of relatively stable, odourless S-alk(en)yl cysteine sulphoxide flavour precursors by the enzymes alliinase and lachrymatory-factor synthase. These secondary metabolites are S-methyl cysteine sulphoxide (MCSO, methiin; present in most Alliums, some *Brassicaceae*), S-allyl cysteine sulphoxide (ACSO, alliin; characteristic of garlic), S-*trans*-prop-1-enyl cysteine sulphoxide (PECSO, isoalliin; characteristic of onion) and S-propyl cysteine sulphoxide (PCSO, propiin; in onion and related species). Information from studies of the transformation of putative biosynthetic intermediates, radiolabelling and from measurements of sulphur compounds within onion and garlic have provided information to suggest a biosynthetic pathway. This may involve alk(en)ylation of the cysteine in glutathione, followed by cleavage and oxidation to form the alk(en)yl cysteine sulphoxide flavour precursors. There is also evidence that synthesis of the flavour precursors may involve (thio)alk(en)ylation of cysteine or a precursor such as O-acetyl serine. Both routes may occur depending on the physiological state of the tissue. There are indications from the effects of environmental factors such as the availability of sulphur that control of the biosynthesis of each flavour precursor may be different. Cysteine and glutathione metabolism are discussed to indicate parallels with Allium flavour precursor biosynthesis. Finally, possible avenues for exploration to determine the origin *in planta* of the alk(en)yl groups are suggested.

P7.18 Diagnosis of plant sulphur status

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Farmers need to have reliable information about the sulphur nutritional status of their crops in order to obtain high yields and to produce high quality foodstuff. The lack of accuracy and precision (sulphate or HI based soil tests, highly variable mobile S compounds and metabolic derivatives in plant tests), a too short time between sampling and result reporting (plant analysis), and high spatial variability of S supply under field conditions, make soil and plant testing unsuitable for fertilizing purpose. Modelling S-supply proved meanwhile to be the most suitable and reliable method for the purpose of fertilizer management. The diagnosis of mineral nutrient status in farming systems always targets for a yield which is some way in the future. Therefore the critical values for target yields always carry the uncertainty of any prognosis which increases with the time lag between sampling and harvest. In physiological research on S, not only the nutritional status of the plants for S, but also for other mineral elements, is often only very poorly defined if at all. Imbalanced mineral nutrition may cause stress situations for metabolism yielding artificial results of experiments. This is of special interest when scaling

up the results of pot experiments under greenhouse or growth chamber conditions to the field level. This contribution compares different methods for the diagnosis of the S status of plants under field and experimental conditions and discusses the need for a better description of the mineral composition of plants in nutritional experiments.

P7.19 Interactions between sulphur and selenium nutrition

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The average diet in many countries does not provide sufficient Se to the population. Agronomists aim to increase the Se content of crops through Se-fertilization. For this to succeed, it is important to determine the potential for different crops to accumulate Se by characterising how yield and Se content respond to Se-fertilization. In addition, since selenate and sulphate compete for uptake by plants, the effect of S-fertilization on these responses must also be determined. Plant breeders are developing crop genotypes with improved Se accumulation and tolerance traits. This strategy may benefit from knowledge of the genes that impact on Se accumulation and tolerance. *Arabidopsis thaliana* has been used to identify these genes. *Arabidopsis* has been grown on agar containing various concentrations of selenate and sulphate to determine how interactions between selenate and sulphate affect growth and shoot concentrations of Se and S. These studies indicate that several transport proteins, with contrasting selectivities, mediate the uptake of selenate and sulphate into plants, and that the relative activities of these transporters are governed by plant nutritional status. They also indicate that Se toxicity is directly related to the Se/S concentration ratio in the shoot. A screen for Se tolerance was devised to obtain mutants lacking root selenate/sulphate transporters and/or enzymes involved in the reduction and assimilation of selenate.

P7.20 Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants

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Dimethylsulphoniopropionate (DMSP) is produced in high concentrations in many marine algae, but in higher plants only in a few salt marsh grasses of the genus *Spartina*, in sugar cane (*Saccharum officinarum*) and in

the Pacific strand plant *Wollastonia biflora*. The high concentrations found in higher plants (up to 250 $\mu\text{mol g}^{-1}$ dry weight) suggest an important role, but though many functions have been suggested (including methylating agent, detoxification of excess sulphur, salt tolerance and herbivore deterrent), its actual functions remain unclear. The fact that the ability to produce DMSP in high concentrations is found in species that have no taxonomic or ecological relationship suggests that the compound evolved independently and serves different functions in different plants. This is supported by observations that DMSP in *Wollastonia biflora* behaves differently from that in *Spartina* species. While DMSP concentrations in *Wollastonia biflora* have been found to increase with increasing salinity, suggesting a role in osmotic control, such a relationship has not been found for DMSP in *Spartina* species. Recent observations in our laboratory on tissue culture showed that while undifferentiated tissue of *Wollastonia biflora* produced DMSP, such material of *Spartina alterniflora* did not. Our ongoing studies with tissue culture of both species have opened up new avenues of research on DMSP in higher plants, ultimately to elucidate the functions of this enigmatic compound.

P7.21 The role of soil microbes in plant sulfur nutrition

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Only a very small proportion of the sulfur present in agricultural soils is in form of inorganic sulfate. Chemical and spectroscopic studies have shown that most soil sulfur (>95%) is present as sulfate esters or as carbon-bonded sulfur (sulfonates or amino acid sulfur). Plant sulfur nutrition depends on uptake of inorganic sulfate, and yet recent research has demonstrated that, surprisingly, the sulfate ester and sulfonate-pools of soil sulfur are also plant-bioavailable. This overview will summarize the available evidence showing that cycling of soil sulfur between carbon-bonded sulfur, sulfate esters, and inorganic sulfate is catalysed by soil microbes. Microbes are responsible for rapid immobilization of sulfate first to sulfate esters and subsequently to carbon-bound sulfur, but they also catalyse the mineralization of bound forms of sulfur in the soil, releasing inorganic sulfate for utilization by plants. The rate of sulfur cycling depends very strongly on the microbial community present, and on its metabolic activity, though it is not yet known if specific microbial species or genera control this process. The genes involved in mobilization of sulfonate- and sulfate ester-sulfur by one common rhizosphere bacterium, *Pseudomonas putida*, have been investigated. Mutants of this species that are unable to transform sulfate esters show reduced survival in the soil, indicating that sulfate esters are important for bacterial S-nutrition

in this environment. *P. putida* mutants that cannot metabolize sulfonate-sulfur are also deficient in plant growth promotion, suggesting that the ability to mobilize bound sulfur for plant nutrition is an important role of this species.

P7.22 Molecular bases of the sulfur-induced resistance concept in plant-pathogen interactions

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The co-evolution of plants and their bacterial and fungal pathogens resulted in the development of numerous innate and inducible defense mechanisms such as phytoalexins and antifungal toxins. These compounds often contain reduced sulfur in functional groups that are essentially donated from the amino acids cysteine or methionine. Prime examples are the sulfur-rich peptides of the thionin and defensin family and several secondary metabolites. Consequently, optimal plant sulfur nutrition is required to provide reduced sulfur for the formation of these defense compounds. Therefore, the recent discovery of enhanced tolerance of crop plants to fungal pathogens based on optimal sulfate supply provides a new approach to improve plant health and yield. An axenic pathosystem consisting of *Arabidopsis thaliana* and either the necrotrophic fungus *Alternaria brassicicola* or the defense signal methyljasmonate has been established that allows precisely defined nutritional conditions. The experiments aim at the identification of candidate metabolites and genes that co-ordinately respond to pathogen stress and optimal sulfate supply, but not to either condition or sulfate deficiency alone. This approach will lead to the dissection of this phenomenon of quantitative resistance and eventually to the understanding of the underlying mechanisms. Analysis of sulfur metabolites and cDNA arrays indicate that indeed key steps in sulfate reduction, sulfur amino acid biosynthesis and glucosinolate formation are up-regulated in response to induction under the condition that optimal sulfate nutrition is provided.

P7.23 Elemental sulphur as an induced antifungal substance in plant defence

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Man's oldest fungicide has probably long functioned in this role in plants, as a natural component of induced antifungal defence. Elemental sulphur (S^0) is the only inorganic phytoalexin and the only phytoalexin produced by so many different taxa. S^0 (detected by GC-MS as $^{32}S_8$) is produced in representative spp. of

Sterculaceae (cacao), Solanaceae (tomato, tobacco), Malvaceae (cotton) and Leguminosae (French bean) in response to xylem-invading fungal and bacterial pathogens. Production was more rapid and intensive in disease resistant genotypes. Gene expression for S^0 production may be xylem-specific as S^0 was not present in leaves of six spp. undergoing hypersensitivity to *Pseudomonas syringae*. Anomalously, high constitutive S^0 levels occurred in leaves of *Arabidopsis* and *Brassica oleracea*. S^0 was highly toxic (ED50 0.8–3 $\mu\text{g/ml}$) to many fungal pathogens representing ascomycetes, basidiomycetes and deuteromycetes, but not to an oomycete, *Phytophthora*, or to bacteria. Levels in tomato xylem and *Arabidopsis* leaves were potentially inhibitory, but in other interactions were below theoretically toxic concentrations. However, S^0 accumulation is highly localized, suggesting the element is produced in sufficient amounts, at the right time and place to be effective. SEM-EDX revealed S in tomato and cacao xylem walls, xylem parenchyma and vascular gels, all sites appropriate to counter vascular pathogenic *Verticillium dahliae*. Transient increases in sulphate, glutathione and cysteine occurred in tomato xylem. The sulphate may reflect over-expression of sulphate transporters, but the thiols might be possible precursors. Analysis of differential gene expression should reveal what may be a novel biosynthetic pathway of S^0 formation in eukaryotes.

P7.24 withdrawn

P7.25 Effect of cadmium on H^+ ATPase activity of plasma membrane vesicles isolated from roots of different S-supplied maize (*Zea mays* L.) plants

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Cadmium can be readily taken up and accumulated by vascular plants. The plasma membrane of root cells constitutes the major membrane barrier between cytoplasm and soil environment and is the first functional site of contact of the root with any ion, so this may have a number of consequences for heavy metal toxicity. In fact, plasma membrane contains potential metal-sensitive enzyme systems, like H^+ ATPase. It is well known the role of sulphide in plant responses to Cd: chelation of metal by high-affinity ligands, such as phytochelatin (PCs), seems to have a primary and direct role in defensive response. This observation gives the rationale for studying the interactions between sulphur availability and Cd exposure. Maize plants were grown for 10 days in nutrient solution. Then, half of the plants were S-deprived and half of the plants of each treatment (+S and -S) were supplied with 100 μM Cd. Roots were collected 0, 1, 2, 3 and 4 days from the beginning of treatment and used for chemical analysis and enzyme

assays. In this work we report on the alteration of non-protein thiols levels and on plasma membrane H^+ AT-Pase activity, by measuring changes in its phosphohydrolytic activity and H^+ pumping capacity, in Cd exposed and differently S supplied maize roots. Cadmium showed a differential inhibiting effect on proton transport activity and phosphohydrolytic activity, the most pronounced one on ATP-dependent H^+ -accumulation, suggesting thus that the metal would interfere with permeability membrane properties. Cadmium inhibitory effect was still more pronounced in S-deficient maize plants.

P7.26 Molecular and biochemical characteristics of sulfur-starved tobacco plants

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Plants possess high physiological flexibility and ability to adjust their metabolism to the conditions of the environment. Higher plants are capable of sulfur assimilation, therefore regulatory mechanisms must exist that coordinate the demand with the availability of sulfur. To elucidate this regulatory network, we have applied subtractive hybridization method to tobacco plants subjected to 48 hours of sulfur starvation in comparison to non-stressed plants. Before treatment, plants were grown on sulfur-sufficient hydroponics medium for two months. The material, harvested separately from four parts (young leaves, mature leaves, stems and roots), was pooled from ten plants per condition to minimize the effects of biological variation. The levels of sulfur-related metabolites (total sulfur, sulfate, thiols, glutathione and ascorbate) were assayed. We noticed most significant changes in the young leaves fraction; therefore, we decided to choose this material to perform suppression subtractive hybridization. A small fraction of obtained cDNAs clones was subsequently screened on the macroarrays to select true positives. After three rounds of hybridizations, 18 up- and 11 down-regulated clones were identified and their differential expression was verified by Northern blots or semi-quantitative RT-PCRs. Sequencing of the clones revealed their homology to the number of previously reported genes, encoding proteins involved in energy metabolism, general stress response, protein degradation and also several unknown cDNAs. Our challenge is now the identification of *cis*- and *trans*-acting elements responsible for regulation of gene expression during sulfur deficiency and the investigation of the unknown cDNAs identified during experiment.

P7.27 Lignification of young maize plants under sulphate deprivation

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Young maize (*Zea mays* L., Poaceae) plants were grown in a complete, well-oxygenated nutrient solution and then were subject to deprivation of their external source of sulphate for 12 days. CoASH is required for the transformation of p-coumaric acid to p-coumaryl-SCoA, while S-adenosyl-methionine (SAM) is used for the methylation process during biosynthesis of ferulic and sinapic acids. As these are key transformations of the lignification process, we hypothesized that the process is affected during the treatment. Thus, we monitored the changes in the lignification of leaf and root free hand cross sections *in situ* by staining with phloroglucinol, which provides an orange to deep purple colour in the lignified areas, depending on the degree of lignification in the light microscope, and by visualizing autofluorescence of the cross sections in a fluorescence microscope using UV filter with excitation wavelength at 365 nm and blue filter with excitation wavelength at 450–490 nm.

The sulphate deprivation treatment affected leaf and root lignification. The lamina of the fully expanded 2nd leaf of sulphate-deprived plants (–S) presented a more developed lower sclerenchyma and an intense lignification compared to the control at day 6, mainly in the epidermal cells above the lower sclerenchyma as well as in the vascular bundles (VBs). In the lamina of the expanding 4th leaf of –S plants, VBs were more developed, with larger and more xylem vessels compared with the control. In roots, lignins were found only in endodermis cells and especially in –S roots and the width of Casparian strip was 18% and 67% thicker compared with the control, at days 6 and 12, respectively.

P7.28 Anion channels in roots – a role in sulphate transport

E. Diatloff, R. Brown and S.K. Roberts (Lancaster University)

The efflux of both organic and inorganic anions from plant roots plays an important role in plant nutrition. We are using the patch-clamp technique to investigate anion channel activity in the peripheral cells of *Arabidopsis* roots which are at the soil:rhizosphere interface. Plant were grown on luxurious sulphate supply (1.7 mM) for 7–10 days and the patch clamp technique was applied to root protoplasts derived from the epidermal cells of the elongation zone and young root hairs. To study sulphate-efflux currents, the pipette was loading with a

medium containing 25 mM Cs₂ sulphate, 1 mM MgATP, 5 mM EGTA, 10 mM HEPES, pH 7.2, and the protoplast bath solution contained 0.5 mM LaCl₃, 10 mM CaCl₂, 5 mM MgCl₂, 10 mM MES, pH 6.0. In the whole-cell configuration, all cells examined showed sulphate efflux currents which were time-dependent, inward rectifying and showed an average maximum peak current density of approximately 30 ± 3 pA/pF at approximately -90 mV. These currents could be partially blocked by the anion channel blocker niflumic acid. Reducing the pipette sulphate concentration to 1 mM reduced the sulphate efflux currents to 8 ± 2 pA/pF. These results indicate that sulphate efflux does occur from root cells of *Arabidopsis* and that this transport is most likely to be conducted by anion channels. It has been shown that when plants are grown in luxurious supply of nitrate or sulphate where influx greatly exceeds demand, then efflux from roots can be as high as 70–80% of the influx (1, 2). The presence of sulphate-efflux channels together with the high influx through sulphate transporters suggests extensive cycling of sulphate at root plasma membrane. Sulphate-efflux channels could play a role in the homeostasis of cellular sulphate concentrations and may limit the usefulness of over-expression of sulphate transporters without a sink for sulphate.

References:

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P7.29 The impact of atmospheric nitrogen deposition on sulfur nutrition in *Brassica oleracea* L.

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Inorganic carbon, reduced nitrogen and sulfur are required for protein synthesis, therefore the coordination between the assimilatory pathways of nitrate and sulfate is necessary to meet the demand of amino acids available for protein synthesis. The mutual regulation of both pathways has been demonstrated in cultured cells systems. Whether the same kind of regulation occurs at a whole plant level remains an open question. The use of NH₃ as atmospheric reduced nitrogen source and the manipulation of the pedospheric nitrogen and sulfur supply is, to our knowledge a new approach in regulatory studies in both metabolic pathways. The aim of our research is to 1) obtain more insight into interrelated regulatory aspects of uptake and assimilation of nitrogen and sulfur, under steady state conditions at 'a whole plant level', and 2) to assess the significance of the

impact of atmospheric nitrogen (NH₃) deposition at sufficient as well as limiting pedospheric nitrogen and sulfur supply. This poster presents the results of a series of experiments with *Brassica oleracea* (curly kale) seedlings, which were exposed to 0, 4 and 8 μl l⁻¹ of NH₃ for two weeks, under sulfur sufficient and sulfur deprived conditions. Growth, changes in biochemical composition and nitrate and sulfate uptake rates were determined.

P7.30 Secondary sulfur compounds in onion: an infinite sink for reduced sulfur?

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Atmospheric hydrogen sulfide (H₂S), a phytotoxic gas, can be used as sulfur source for plant growth. It is taken up by the stomates, directly incorporated as sulfide into cysteine and subsequently into other organic sulfur compounds. H₂S exposure, in combination with variations in sulfate nutrition, can be used as a tool to study sulfate uptake and reduction and the interaction between shoot and roots. Onions (*Allium cepa* L.) synthesize large amounts, up to 80% of the total sulfur content, of sulfur containing secondary compounds (alliins and γ-glutamyl peptides), that are the precursors for the characteristic flavor compounds of onions. After a 7-day fumigation with 0, 0.15, 0.3 and 0.6 μl l⁻¹ H₂S, the total sulfur content in shoots of onion was doubled. This was partly caused by an increase in non-protein organic sulfur compounds, presumably alliins and/or γ-glutamyl peptides, as concluded from a strong decrease in the organic nitrogen/sulfur ratio. An increase in the sulfate content, which can be explained by direct oxidation of H₂S, the breakdown of organic sulfur compounds or by a decrease in the reduction of sulfate, caused the other part. The role of secondary compounds as sink for reduced sulfur and the impact of H₂S exposure on the uptake and assimilation of sulfate will be discussed.

P7.31 Sulphur nutrition of poplar during flooding

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Anoxic conditions caused by flooding of the flooding tolerant species poplar (*Populus tremula* x *P. alba*) influenced sulphur nutrition of the whole tree. In the roots, activity and transcript of APS reductase, the highly regulated key enzyme of the sulphate assimilation pathway, completely disappeared, but the Cys content increased. A higher glutathione content of phloem exu-

dates after flooding seems to indicate an increased transport of reduced sulphur to the roots. Still the export rate of ^{35}S -sulphur out of mature leaves after flap feeding ^{35}S -sulphate was not different between flooded and control poplar trees. Since the only allocation to the basipetal bark was enhanced but allocation to the roots was diminished, enhanced phloem exudate contents of GSH may indicate a reduced sink rather an enhanced source strength in response to flooding. Hence an elevated transport of GSH could not be responsible for the higher Cys content in the roots. Since protein contents were unaffected, the higher Cys content in roots could also not originate from protein breakdown. Enhanced O-acetylserine(thiol)lyase activity was measured in the roots in response to flooding demonstrating an apparent uncoupling of sulphate reduction and Cys synthesis. A possible contribution of cysteine synthesis in detoxification of sulphide produced in the anoxic soil is discussed.

P7.32 ‘Smart Plant’ technology for sensing sulfur deficiency in winter wheat

J.R. Howarth, P. Barraclough and M.J. Hawkesford, Rothamsted Research, Harpenden, UK

We aim to identify novel plant genes whose expression are specifically regulated by sulfur(S)-deficiency and will use the promoters controlling the expression of candidate genes in a reporter system for diagnosing sulfur status of winter wheat. Leaf samples from plots on Rothamsted’s field experiments have been used to compare wheat gene expression profiles under various nutrient-deficient conditions (N, P, K, S, Mg). Differences in gene expression between plants from trial plots have been compared by cDNA-AFLP and microarray analysis and initial screens have detected putative genes specifically responsive to S limitation. Specificity of candidates has been verified by large scale reverse-northern primary screening. Regulated genes from the primary screen were analysed for quantitative expression in relation to differential nutrient status by northern blotting. The promoter regions of genes with nutrient-regulated expression are being isolated. The promoters will then be coupled to a reporter gene and be used in a transient expression assay to monitor nutritional status following re-introduction of the ‘reporter’ construct by biolistic transformation. Specific deficiency-induced promoters coupled to appropriate reporter genes will be suitable for a number of diagnostic applications. Such ‘Smart Plant’ technology will aid precision farming, avoiding environmentally-damaging excessive application of fertilisers.

P7.33 Microarray analysis of wheat grain transcriptome: sulphur deficiency

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Sulphur in UK soils has declined dramatically over the last ten years making sulphur deficiency an increasingly common problem, resulting in decreased crop quality, yield and nitrogen losses. The Broadbalk winter wheat experiment at Rothamsted is the longest running fertiliser experiment in the world and provides an invaluable resource for wheat genomic studies. Since 2000, a treatment was modified to test for sulphur deficiency, by replacing potassium sulphate fertiliser with potassium chloride. We compared this plot with a control (adequate S-nutrition) as the source for materials at two stages of grain development. Transcriptome profiling was performed using the IGF 10 000 wheat unigene set (<http://www.cerealsdb.uk.net/igf.htm>), which represents a substantial proportion of whole wheat genome. This was created from 26 different wheat EST libraries. Analysis of the microarray data using GeneSpring software and real-time RT-PCR has revealed at least 7 genes which are highly up-regulated in the S-deficiency treatment, suggesting that these genes may be used for genetic markers for S-deficiency status. Furthermore, many genes that participate in sulphur uptake, metabolism, transport and starch synthesis have been identified. In addition there were many up-regulated genes of unknown function plus several potential regulatory factors. Further analysis, of the results will lead to a better understanding of plant responses to the genetic regulation of sulphur deficiency.

P7.34 Sulfate and chromate uptake in *Brassica juncea*: synergy or competition?

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Chromium contamination of the environment can be originated by waste products of several industries. In particular, the presence of the high carcinogenic hexavalent form (chromate) is of major concern. Cr(VI) is supposed to be taken up by plants through an active mechanism mediated by sulfate carriers. In order to understand the effect of the presence of Cr(VI) on sulfate uptake, *Brassica juncea* plants were conditioned for 7 days with different concentrations of sulfate (0.2 mM and 1.0 mM) and chromate (0.2 mM and 1.0 mM). A growth reduction of roots and shoot was observed already after 2 days of treatment in plants grown in presence of Cr, independently from sulfate and chromate

concentrations. Only after 7 days of Cr treatment a lower foliar pigment content was observed. The presence of both 0.2 mM and 1.0 mM Cr(VI) did not affect sulfate uptake rates in plants grown in 0.2 mM SO_4^{2-} . On the contrary, sulfate uptake rates were enhanced in plants supplied with 1.0 mM SO_4^{2-} after 4 days of 1.0 mM Cr(VI) treatment. The same amount of Cr was accumulated in roots and shoot of plants grown with 0.2 mM chromate and either 0.2 mM or 1.0 mM SO_4^{2-} . Higher Cr content was observed in plants treated with 1.0 Cr, with no difference due to sulfate availability.

P7.35 Distribution of plant gamma-glutamyl-transferase at subcellular and tissue level

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Gamma-glutamyl transpeptidase (GGT) is an ectopeptidase that catalyzes the transfer of the gamma-glutamyl moiety of gamma-glutamyl peptides to other amino acid and peptide acceptors as well as the hydrolytic cleavage of the gamma-glutamyl group of donor peptides. Although thoroughly studied in animal science, in plants GGTs have been poorly characterized and their role probably underestimated. By means of an enzyme-histochemical procedure, we localized GGT activity in different tissues from some plant species. Our results show that GGT activity has an uneven distribution and is localized in the parenchymal cells of conductive tissues closely associated with vascular bundles, but also in stomata, epidermal cells and root tips. An antibody directed against a synthetic peptide corresponding to the last 20 aminoacids of the heavy subunit of human GGT was used for immunoprecipitation of proteins from *Arabidopsis thaliana* and *Zea mays* leaf extracts. The immunocomplex catalyzes the release of p-nitroaniline from the synthetic substrate gamma-glutamyl-p-nitroanilide, a typical reaction used to assay GGT activity. The activity was inhibited by serine/borate, a known competitive inhibitor of GGTs. By means of SDS-PAGE we determined the molecular weight of the immunocomplexed proteins. The same antibody was used for immunocytochemical analysis by electron microscopy. This enabled us to visualize the GGT protein in plasma membranes and cell walls; GGT can therefore be properly defined as an apoplastical enzyme.

P7.36 The impact of artificially elevated glutathione-concentrations on symptom development in virus-infected *Cucurbita pepo* L.

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Infection of cucurbit plants by viruses often results in oxidative stress leading to a reduction in plant growth, yellowing, mosaic and blistering of the leaves, and fruit malfunction resulting in severe crop losses every year. Infected-plants use various antioxidative defence reactions to avoid excessive oxidative damage. The ascorbate-glutathione cycle is known to play significant roles in the detoxification of reactive oxygen species generated by viruses within plants. Glutathione works synergistically with other cellular antioxidants in order to neutralize and scavenge oxygen and other free radical species, thereby preventing or diminishing oxidative damage. We performed various experiments to clarify, whether compatible *Zucchini yellow mosaic virus* (ZYMV)-infection lead to changed glutathione-levels in Styrian pumpkin plants (*Cucurbita pepo* L. *subsp. pepo* var. *styriaca* Greb.). Additionally, pumpkin seedlings and callus cells were treated with different concentrations of 1) the cysteine precursor OTC (L-2-oxothiazolidine-4-carboxylic acid), which is described to lead to strong increases of glutathione- and cysteine- contents; and 2) salicylic acid that acts as a component of the signal transduction system, which plays important roles in defence mechanisms during pathogen-attack. In the present experiments pre-treated plants as well as regenerated plants from callus cultures showed enhanced glutathione-levels directly after the treatments and suppressed and delayed symptoms three weeks after the infection with ZYMV.

P7.37 Structure-function analysis of the C-terminal domain of the *Arabidopsis thaliana* Sultr1.2 sulfate transporter

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Amino acid sequence alignment of the 14 putative sulfate transporters identified in *Arabidopsis thaliana*, shows a high homology for 12 among 14, notably in their carboxyl-terminal cytoplasmic regions which contain about 120 amino acids. Bioinformatic analysis predicts the presence in this C-terminal region of a STAS domain (Sulfate Transporters and AntiSigma antagonists) which seems to be consensual in many other sulfate transporters of very diverse organisms. We focus on

studying the SULTR1.2 *Arabidopsis thaliana* high-affinity sulfate transporter by generating several structural modifications using site directed mutagenesis and progressive deletions in the protein C-terminal region. Functional analysis of all modified constructions, have been performed by heterologous functional complementation of a yeast mutant defective in its sulfate transport capacity by measuring the effects of the mutations on the yeast doubling time and on their ability to transport ^{35}S -sulfate. Several key modifications of the SULTR1.2 transporter structure will be described.

P7.38 Cd induced changes in sulphur metabolism of mosses – a specific stress response

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Plants are able to cope with a heavy metal polluted environment by a strict regulation of the cellular metal homeostasis, which includes the biosynthesis of heavy metal binding substances, subcellular metal compartmentation and adapted metabolism. The enzymatic synthesis of complexing thiol peptides (e.g. phytochelatin) is a fundamental defense mechanism against heavy metals in higher plants. Our research has shown a singular effect of heavy metals in mosses: an increase of the glutathione (GSH) pool, which is in contrast to higher plants. *Physcomitrella patens* was exposed in liquid culture with up to 10 μM Cd^{2+} in order to investigate the regulation of enzymes involved in assimilatory sulfate reduction pathway by use of real-time PCR. Cd^{2+} lets increase all transcripts of tested genes involved in sulfate assimilation. *P. patens* showed 4 times more products of genes coding for sulfate transporter, for sulfite reductase and for phosphoadenosylphosphosulfate reductase within 3 d. In addition, a doubling of intracellular cysteine and GSH content was noted, while the moss did not produce phytochelatin. These results suggest an activation of the assimilatory sulfate reduction pathway by increasing transcription of related genes to enhance GSH biosynthesis as the remarkable stress response.

P7.39 Response of two cultivars of Chinese cabbage to elevated atmospheric SO_2

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Previous experiments under field conditions in China with two cultivars of Chinese cabbage (Kasumi F1) and a local cultivar (Beijing 3) showed that 'Beijing 3' was more sensitive to sulfur deficiency than 'Kasumi F1'.

Both cultivars responded to application of fertilizer S with increases in shoot biomass, and increases in S and N content. The local cultivar 'Beijing 3' needed twice as much kg S ha^{-1} as 'Kasumi F1'. The response of these two cultivars to atmospheric SO_2 was studied by exposing the plants to a range of SO_2 levels (0.10–0.80 $\mu\text{l l}^{-1}$) under controlled environmental conditions, and measuring the effects on shoot biomass and organic and inorganic S and N compounds. In these experiments pedospheric nutrient supply was not limiting. The results showed differences in response between the cultivars. Shoot fresh weight of 'Kasumi F1' was not affected upon exposure to 0.10 and 0.20 $\mu\text{l l}^{-1}$ SO_2 , while shoot fresh weight of 'Beijing 3' was reduced at these concentrations. Shoot growth of both cultivars was reduced by exposure to SO_2 levels higher than 0.20 $\mu\text{l l}^{-1}$. Shoot growth of 'Beijing 3', therefore, was more sensitive to atmospheric SO_2 than that of 'Kasumi F1'. In both cultivars the contents of total S, sulfate and water-soluble non-protein thiol compounds were increased. A large proportion of total S was present as sulfate and SO_2 exposure did not affect the ratio of organic S to total S. In both cultivars organic N content was unaffected by exposure to SO_2 . In 'Beijing 3', however, nitrate content was increased upon exposure to SO_2 levels of 0.40 $\mu\text{l l}^{-1}$ and higher, resulting in a higher total N content.

P7.40 Changes of the subcellular distribution of glutathione during virus-attack in *Cucurbita pepo* L.

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The tripeptide glutathione is involved in various important cell processes including the transport and storage of sulfur, the detoxification of xenobiotics and the compensation of oxidative stress evolving from various abiotic and biotic stress situations. During pathogen-attack, elevated glutathione-concentrations are thought to protect plants against reactive oxygen species, which are formed within plants to defeat the dangerous invader (hypersensitive reaction). Since most of the data concerning glutathione-contents within pathogen-infected plants are gained from whole organs by using biochemical methods, little is known about changes in the distribution and localization of glutathione within single cells during pathogen-attack. Such data would be needed to speculate about the mechanisms behind the generally observed protective role of glutathione during pathogen-infections.

In the present study a high resolution immunogold labeling method was used to study changes in the distribution and localization of glutathione during *Zucchini yellow mosaic virus* (ZYMV)-infection in *Cucurbita pepo* L. *subsp. pepo* var. *styriaca* Greb. (Styrian pumpkin). Sta-

tistical evaluation of the amount of gold particles bound to glutathione revealed an increase of glutathione-contents within the cytosol (3 fold), nuclei (2.1 fold), peroxisomes (1.8 fold) and plastids (1.5 fold) in virus-infected younger leaves whereas mitochondria showed slightly decreased glutathione-levels when com-

pared to the control. Older virus-infected leaves showed an increase in glutathione-contents (between 1.2 to 1.7 fold) within all investigated organelles except plastids. Within virus-infected root tip cells decreased amounts of glutathione were found in all organelles of about 0.8 fold when compared to the control.