

affected by the plant sulfur supply/status. In addition to sulfate taken up by the roots, *Brassica* species are able to utilize atmospheric sulfur gasses, viz. H<sub>2</sub>S or SO<sub>2</sub> taken up by the leaves as sulfur source for growth. In the current study the impact of H<sub>2</sub>S or SO<sub>2</sub> on glucosinolate metabolism was studied in two *Brassica* species characterized by a high (*B. juncea*) and low (*B. rapa*) glucosinolate content. 10-day-old seedlings were grown on a 25% Hoagland nutrient solution containing 0.5 mM sulfate for 3 days and subsequently transferred to fresh 25% Hoagland solution at 0 mM sulfate (-S) or 0.5 mM sulfate (+S) and exposed to 0.25 µl l<sup>-1</sup> H<sub>2</sub>S or SO<sub>2</sub> for 7 days. At an ample sulfate supply, exposure of both species to H<sub>2</sub>S or SO<sub>2</sub> hardly affected content and composition of glucosinolate. H<sub>2</sub>S or SO<sub>2</sub> exposure resulted in a slight decrease in expression of APS reductase expression whereas that of APS kinase and ATP sulfurylase remained unaffected. Sulfate-deprivation of plants resulted in a decreased biomass production and glucosinolate content. Expression of APS reductase was strongly enhanced in sulfate-deprived plants but expression of both APS kinase and ATP sulfurylase hardly changed. When sulfate-deprived plants were exposed to H<sub>2</sub>S or SO<sub>2</sub>, plant growth was restored, however, the glucosinolate content remained lower than that of sulfate-sufficient plants. Moreover, the expression of APS reductase was partially down-regulated again, whereas expression of APS kinase and ATP sulfurylase remained unaffected.

### 8) Effect of S-limitation on osmotic potential components in oilseed rape leaves: towards the development of early indicators?

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Oilseed rape (*Brassica napus*) is a S demanding crop, S-limitation resulting in a reduction of yield and of nutritional quality of seeds. Optimization of S fertilization requires to identify indicator of S nutrition that could be used early in the growth cycle.

In this study, we examined the effects of S-limitation on osmotic potential components of oilseed rape leaves. Plants were grown at vegetative stage and submitted to S-limitation (S+: 508.7 µM SO<sub>4</sub><sup>2-</sup> or S-: 8.7 µM SO<sub>4</sub><sup>2-</sup>) and were harvested at 0, 1, 2, 3, 7, 13, 24 and 34 days of S-limitation. Each plant was sampled as old leaves, new leaves emerging after S-limitation, roots and petioles.

Plant growth was maintained during the first 13 days of S-limitation as a result of massive internal sulfate mobilization mostly from leaves and its subsequent assimilation. This was at least partly compensated for by an accumulation of malate, nitrate, chloride and phosphate in leaves and to a lesser degree in roots. More surprisingly, leaf osmotic potential decreased after two days of S-limitation. Other compounds such as amino acids, soluble sugars and cations will be quantified in order to evaluate their contribution to the leaf osmotic potential. Additional data (<sup>15</sup>N-nitrate uptake, nitrate reductase activity, transcript levels of sulfate and nitrate transporters) show that under

S-limitation, osmotic potential is affected earlier than growth or N metabolism suggesting that field measurements of leaf ion contents, acting as osmoticum, could be used as early indicators for S fertilization management.

### 9) Elucidating the molecular components that allow *Salicornia* and *arcocornia* to thrive in high sulfate and sulfide levels

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*Salicornia* and *Sarcocornia* are extreme halophytes that grow on sea water with high sulfide and sulfate levels and have been recently introduced as new crops for extreme salt conditions such as in the level of Dead Sea water. We are exploring the mechanism/s that allows these plants to cope with sulfate and sulfide levels, which are toxic to many other plants. We grew the plants at different NaCl, sulfate and sulfide levels and we are scanning for relevant genes and proteins that might be related to plant resistance to those stresses.

### 10) The role of sulfite reductase in sulfite homeostasis

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Using *Arabidopsis* and tomato plants with modified SiR expression, we investigated the role of SiR in various metabolic processes in plants. We observed that resistance to ectopically applied SO<sub>2</sub>/sulfite was a function of SiR expression levels and that plants with reduced SiR expression exhibited higher sensitivity than the wild type. The sulfite sensitive mutants accumulated sulfite and showed a decline in glutathione levels. In contrast, mutants that over-express SiR were more resistant to sulfite toxicity; exhibiting little or no damage. Resistance to high sulfite application was manifested by fast sulfite disappearance and increase in glutathione levels. The notion that SiR plays a role in the protection of plants against sulfite was supported by the rapid up-regulation of SiR transcript and activity within 30 min of sulfite injection into *Arabidopsis* and tomato leaves. Our results indicate that, in addition to participating in the sulfate assimilation reductive pathway, SiR also plays a role in sulfite homeostasis together with sulfite oxidase and the other members of the sulfite network.

### 11) Regulatory Network of SO<sub>2</sub> detoxification

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Abiotic stress occurs when plants are exposed to high concentrations of sulfur dioxide (SO<sub>2</sub>) and its derivative sulfite. Physiological studies suggested sulfite oxidase (SO) as one important component of SO<sub>2</sub> detoxification. However, it is unknown to which extent plant fumigation with SO<sub>2</sub> triggers a specific transcriptional response. To address this question, we compared *Arabidopsis* wildtype (WT) and SO knock-out lines (SO-KO) facing the impact of 600 nL L<sup>-1</sup> SO<sub>2</sub> using RNAseq to quantify absolute transcript abundances. These transcriptome data were correlated to sulfur metabolism related enzyme activities and metabolites obtained from the same samples in a physiological study. SO-KO plants showed first symptoms of leaf injury after fumigation that were not detectable in fumigated WT plants. Analogously, SO-KO exhibited remarkable and broad regulative responses at the mRNA level, especially in transcripts related to sulfur metabolism enzymes but also of those related to stress response and senescence. Our data provide evidence for a highly specific co-regulation between SO and sulfur related enzymes like APS reductase. Moreover, we could show that beside of SO a new player comes into business – apoplastic peroxidase (PRX). PRXs known from work of PFANZ and colleagues in the early 1990ies are able to detoxify sulfite. We could show that under SO<sub>2</sub>-fumigation the mRNA of *prxcb* is 5fold up-regulated. This result implies that sulfite detoxification is of exceptional importance so that the plants have evolved a complex network of mechanisms and enzymes for controlling its level including regulation of stomata, generation of enzymes like SO and PRX for direct oxidation and use of the sulfur assimilation pathway for reductive detoxification.

## 12) The arbuscular mycorrhizal symbiosis influences sulfur starvation responses of *Medicago truncatula*

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Arbuscular mycorrhizal (AM) symbiosis is a mutualistic interaction that occurs between most vascular plants and fungi of the phylum Glomeromycota. The main benefit for both organisms in this relationship is an exchange of nutrients to the plant in return for plant-derived carbohydrates. In the past, the primary focus has been on mycorrhizal phosphate transfer, mainly because low phosphate concentrations in soils are often the main growth-limiting factor and known to be necessary to establish symbiosis. Evidence for symbiotic uptake pathways for additional nutrients emerged from the recent identification of a number of other nutrient transporter genes, which are also specifically expressed or induced in mycorrhized roots. In this study we were interested whether *in vivo* sulfate supply from the fungus to the plant does occur under conditions of sulfate starvation in addition to a mild phosphate starvation. Sulfur starvation leads to typical S starvation phenotype in *Medicago truncatula* Gaertn. with reduced thiol, protein and chlorophyll contents and consequently a reduced biomass in *M. truncatula*.

We investigated whether mycorrhizal colonization by the fungus *Rhizophagus irregularis* (previously termed *Glomus intraradices*) influences leaf metabolite composition and the expression of sulfur starvation-related genes when applying different sulfur and phosphate fertilization treatments to *M. truncatula*.

Single amino acids, as well as S-containing metabolites and ions, were analyzed in shoots of mycorrhized and non-mycor-

rhized plants grown under S starvation (-S) and S repletion (+S) as well as in plants fertilised with 1 mM phosphate (+P), which represents a mild phosphate starvation, and plants grown under strong phosphate starvation (-P). Primary metabolites of the S assimilation pathway like cysteine, methionine, glutathione, gamma-glutamylcysteine, sulfite, and also total protein content were correlated to the sulfate content in shoots. All metabolites displayed strong reductions under -S conditions which were slightly alleviated by mycorrhiza formation. This mainly resulted in increased biomass as additional sulfate was converted into growth. Leaf metabolite concentrations clearly showed that phosphate starvation has a greater impact than sulfur starvation on plant metabolism, with no demand for sulfur at strong phosphate starvation. However, when phosphate nutrition is high enough, mycorrhizal colonization reduces sulfur stress responses, as a result of symbiotic sulfur uptake.

We made use of a two-compartment system in which the plant's roots (in one compartment) were restricted to grow into a compartment, which only the fungus could reach. Application of <sup>35</sup>S to this "fungal compartment", confirmed that sulfate was taken up by the fungal hyphae and transported to the plant detected as radioactivity in the plant's leaves.

Similar to other plant species, sulfate deprivation also leads to the induction of sulfate transporters in *M. truncatula*. In this study nine putative sulfate transporter genes, which correspond well to the known classifications in other species were identified in *M. truncatula*. We also investigated mRNA accumulation levels in leaves and roots of mycorrhized and non-mycorrhized plants grown under the two different S concentrations (+S and -S) and at 1mM phosphate. Sulfur starvation leads to the induction of sulfur starvation-related genes and the mycorrhizal provision of sulfate resulted in a less strong induction of these genes. Thus, the transcripts of marker genes of the S-assimilation pathway, adenosine-5'-phosphosulfate reductase (APR), and of the OAS-cluster gene of the ChaC-like protein were highly induced under sulfate starvation in sulfur starved non-mycorrhized plants while the induction level was lower in sulfur starved mycorrhized plants. It has to be noted that no additional sulfate source was provided but fungal hyphae mobilized sulfate more efficiently from the substrate than plant roots.

In this study we could show that mycorrhizal colonization of sulfur starved plants led to an increased accumulation of biomass and a shift in the metabolite pattern in the direction of that of sulfur-replete plants. We can assume that mycorrhizal colonization is able to reduce sulfur starvation responses in *M. truncatula*. This demonstrated the importance of mycorrhizal sulfur uptake for plant metabolism, when the plant's phosphate status is high enough for the plant to benefit from increased sulfate nutrition.

## 13) Compartment-specific changes in glutathione and ascorbate levels during high light stress in *Arabidopsis*

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Excess light conditions represent a potential danger to the plant as it can lead to the accumulation of reactive oxygen species (ROS) in chloroplasts by overstraining the electron transport chain during photosynthesis and of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in peroxisomes as a result of glycolate oxidation in the photo-respiratory pathway. If not detoxified ROS can leak into the