Examination of the role of sulfate transporters expressed in seeds by using Arabidopsis T-DNA mutants

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7th Workshop on Sulfur Metabolism in Plants

Advances in plant sulfur research including links to agriculture and environment, significance of sulfur in the food chain and regulatory aspects of sulfur metabolism

Warsaw, Poland, 13-17 May 2008

ABSTRACTS

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INVITED TALK
The link between sulfur and acrylamide risk in cereals and potato

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Severe sulfur deprivation causes a dramatic accumulation of free asparagine in wheat grain, to levels up to 30 times higher than those in grain from plants receiving adequate sulfur [1]. This effect has been observed both in plants grown in pots and in plants grown in field trials on soil with poor nutrient retention. The levels of other free amino acids, notably glutamine, also rise, but free asparagine is of particular significance because it is a precursor of acrylamide, a carcinogen and neurotoxin that forms during baking and other high-temperature processes. Flours from sulfate-deprived wheat can contain up to 5200 µg per kg acrylamide after heating at 160 °C for 20 min, compared with up to 900 µg per kg in flours from wheat grown with adequate sulfur. The amount of acrylamide that is formed correlates closely with asparagine concentration [1, 2].

The other precursors for acrylamide formation are reducing sugars such as glucose, fructose and maltose; these react with amino acids at high temperatures in what is known as the Maillard reaction. Sucrose can also participate because at very high temperatuires it undergoes thermal degradation. The Maillard reaction is important for the food industry because while asparagine produces acrylamide other amino acids produce compounds that determine colour and flavour.

In contrast to its effect on wheat, sulfur deprivation causes a reduction in acrylamide formation during the cooking of potatoes [3]. In some but not all varieties it does cause free asparagine levels to increase but free glutamine increases much more, resulting in a decrease in the concentration of asparagine as a proportion of the total free amino acid pool. We hypothesise that in potatoes where, unlike cereal grains, concentrations of sugars are usually limiting in the Maillard reaction, competition between asparagine and other amino acids is a key determinant of the amount of acrylamide that is formed.

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Session I: **Significance of S nutrition and metabolites in agriculture and plant response to environmental stresses**

Sulfur Induced Resistance (SIR): biological and environmentally sound concept for disease control

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Already *Justus von Liebig* (1803 – 1873) addressed the lack of vitality of soils and nonexistent vigor of plants as relevant causes for increased infections of crops by fungal diseases. Environmentally sound methods for disease control imply for instance soil tillage measures, crop rotation, mixed cropping systems and cultivation of resistant varieties. The targeted use of minerals offers yet another possibility to enhance resistance against pathogens. Here, the direct toxicity of nutrients (elemental S, Cu) and indirect impairment by minerals (Si) needs to be distinguished from nutrient-mediated, resistance mechanisms, which were observed for all essential macro and micronutrients, Si and Al [1].
Soil-applied sulfate fertilization proved to significantly reduce infection rate and severity of crops by fungal diseases. The term Sulfur Induced Resistance (SIR) denotes the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes involving sulfur by targeted sulfate-based and soil-applied fertilizer strategies [2]. Metabolic pathways involved in SIR imply for instance the synthesis of phytoalexins, glutathione, glucosinolates and the release of sulfur-containing volatiles. The potential efficacy of SIR expressed as a reduction of the disease index ranged from 5 - 50% and 17 - 35% in greenhouse and field experiments, respectively. Up-to-date research in the field of SIR from molecular to field level is summarized in relation to different host/pathogen systems. In addition, an outlook on research and on-farm implementation of SIR will be given.

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Session I: **Significance of S nutrition and metabolites in agriculture and plant response to environmental stresses**

From seed to cure: aspects of cultivation, preparation and administration of *Tropaeolum majus* L.

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Alternative strategies to stabilize health and performance of livestock animals gained in importance since the ban of antibiotics as feed additives in animal nutrition in the European Union in 2006. Phytopharmaceuticals with a proven efficiency in humans may offer a special prospect in animal nutrition. Different medical herbs such as oregano, clove, thyme, peppermint, fennel, caraway, lemon grass and many others have been tested with respect to their stabilizing or health promoting effects but results proved to be inconsistent [1]. A possible reason for inconsistent findings can be the lack of a quality control, particularly the missing analysis of active ingredients of the herbs. It was the aim of the present study to optimize the quality of *Tropaeolum majus* by adequate cultivation, harvesting and processing techniques, and to investigate the potential of this medical herb as a feed supplement in animal nutrition.
Nasturtium (*Tropaeolum majus* L.) is a herb with a proven antimicrobial activity, which is caused by benzyl-isothiocyanate the degradation product of glucotropaeolin. A non-destructive harvest of leaves in combination with a gentle drying procedure at 40° C proved to deliver the highest concentration of glucotropaeolin.
In an experiment with piglets, direct and graded supplementation of *T. majus* with the feed was performed over a period of five weeks. *T. majus* was supplemented at an upper dosage of 1 g/kg with the feed, equaling 48.7 mg/kg glucotropaeolin, which resulted in a benzyl-isothiocyanate concentration in the urine of up to 16 µmol/L. This concentration ought to be high enough to control a broad range of bacteria. Up to 7.3% of the glucotropaeolin taken up by the animals was excreted as bioactive benzyl-isothiocyanate via the urine. No effect was observed on the intestinal microbiota and supplementation with *T. majus* had also no effect on growth performance of healthy piglets.

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Session I: **Significance of S nutrition and metabolites in agriculture and plant response to environmental stresses**

Influence of sulfur fertilization on the insect inventory in oilseed rape during the vegetation period

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Oilseed rape is a widely grown crop with a high sulfur (S) demand and S-fertilization is a regular measure to satisfy the nutrient demand in S-deficient areas. S-fertilization warrants not only crop productivity and quality, but it is also linked to the resistance of plants against fungal infections and will also affect population dynamics of beneficial insects and pests. For elements such as potassium and nitrogen relationships between the nutritional status of crops and the infestation with insects at different developmental stages were shown, while corresponding studies are strictly limited for S. It was the aim of the present study to determine the influence of the S fertilization on the complete insect inventory of oilseed rape during the entire vegetation period with special view to specialist herbivores.
In two succeeding vegetation periods insects were collected from oilseed rape plots that received 0 and 150 kg ha^{-1} S by employing different methods (sweep net, suction sampler, emergence traps, beating tray, funnel traps and plant dissection). Larvae of *Meligethes* spp., *Dasineura brassicae*, *Ceutorhynchus obstrictus*, *Ceutorhynchus napi* and *Ceutorhynchus pallidactylus* and imagines of the order Homoptera (*Brevicoryne brassicae*), Hymenoptera (*Athalia rosae*), Coleoptera (*Meligethes* spp., *Phyllotret*a spp., *Lema melanopus*, *Ceutorhynchus napi*, *Ceutorhynchus pallidactylu*s, *Ceutorhynchus obstrictus*, *Ceutorhynchus floralis*, *Sitona* spp., *Amara* spp.) and Diptera (*Delia radicum*, *Delia platura*, *Delia florilega*, *Dasineura brassicae*, *Scaptomyza flava*) were sampled employing different methods.
The mineral composition of larvae was determined. For *Meligethes* spp. larvae a close relationship between weight of larva and total S concentration was found; S fertilization had no significant effect on the biomass of the larvae. *Meligethes* spp. comprise beetles, which are a pest before flowering due to feeding on closed buds for assessing pollen and oviposition into buds, however beneficial insects during flowering as they favor pollination. S-fertilization resulted in a decreased population before and an increased population after flowering. In addition, S-fertilization increased the number of some predators (*Staphylinidae*, *Tachyporus* spp. and *Syrphidae*) by enhancing the population of their prey.

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Cysteine as limiting factor for glutathione synthesis during virus infection in plants

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Glutathione synthesis, which takes place in plastids and the cytosol, is a highly compartment specific pathway and relies on the supply of its precursors cysteine, glutamate and glycine in these organelles.
In non-stressed plants cysteine is supposed to be the rate-limiting factor for glutathione synthesis. To gain a deeper insight into possible limitations of glutathione synthesis during pathogen attack glutathione and its precursors were quantified with cytohistochemical methods and transmission electron microscopy in single cells and organelles of leaves and roots in both a highly susceptible and highly tolerant *Cucurbita pepo* hyprid (*styriaca* GREB. and cultivar quine) during zucchini yellow mosaic virus (ZYMV) infection. The susceptible cultivar is characterized by the development of strong mosaic symptoms whereas the tolerant cultivar shows no symptoms on leaves and roots even though virus particles and typical ultrastructural alterations can be found in all cells of leaves (and roots).
During ZYMV-infection glutathione contents were much stronger increased in leaves of the tolerant cultivar than in the susceptible one, indicating that high levels of glutathione play an important role in the development of resistance and tolerance. The weaker increase of glutathione in the susceptible cultivar was found to be caused by low levels of glutathione precursors in glutathione producing organelles. Whereas in younger leaves of the susceptible cultivar low levels of cysteine and glutamate were found to be the limiting factor for glutathione synthesis during ZYMV-infection, low levels of glycine are limiting the availability of glutathione in this organ.
In roots, glutathione contents do not appear to be affected by the availability of glutathione precursors during ZYMV-infection as glutathione precursors remained in general unchanged in both the susceptible and the tolerant cultivar. However, as glutathione contents were strongly decreased in the susceptible cultivar but strongly increased in the tolerant one it seems that the transport of glutathione from the leaves to the roots might have been interrupted by ZYMV-infection in the susceptible cultivar but not in the tolerant one.
Summing up, the present study demonstrated that elevated glutathione contents play an important role in the development or resistance and tolerance and that the availability of glutathione precursors limits glutathione production during ZYMV-infection in the susceptible cultivar.

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Cadmium induced thiol peptides in *Chlamydomonas reinhardtii* strains

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Chlamydomonas reinhardtii is an ubiquitous unicellular green algae of fresh water and soil habitats. Culture collections comprise several wild type strains derived from a single isolate. Cadmium stress is investigated in this organism but not comperatively in different strains.
Cd stress (70-500 µM) caused decreased vitality and growth rates in five *Chlamydomonas reinhardtii* representative strains. Upon addition of 70 µM $CdCl_2$ up to 4.2 µM were accumulated intracellularely. These low Cd concentrations were sufficient to cause significant changes in intracellular thiolpeptide pools. Cys, γ-EC and glutathione (GSH) were analyzed by pre-column biman derivatization. Phytochelatins were quantified by reversed-phase HPLC followed by 5'5-dithiobis-2-nitrobenzoic acid post-column derivatization. PC identification was achieved by ESI-qTOF-MS/MS. Phytochelatin synthesis was accompanied by diminished amounts of glutathione. Interestingly, thiol peptide composition was strain dependent.

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Intracellular cadmium detoxification mechanisms in the moss *Physcomitrella patens*

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Treatment of *Physcomitrella patens* with up to 10 µM cadmium causes upregulation of the content of low molecular weight thiols (cysteine, γ-glutamylcysteine, glutathione), while no phytochelatin synthesis was observed [1]. Protonema cultures exposed to 10 µM cadmium for 3 days accumulated 1.5 µmol Cd per g fw accompanied by a threefold increase in the intracellular glutathione (GSH) content. These results suggest that at least part of the Cd taken up into the cytosol may be detoxified by glutathione through formation of $Cd[GS]_2$ complexes.
To test this hypothesis, protonema cultures were labelled in situ with the non-fluorescent, membrane-permeable dye monochlorobimane (MCB). MCB is preferentially conjugated to GSH by glutathione-S-transferases (GST) in a phase II reaction. The resulting glutathione S-bimane (GSB) is fluorescent and membrane-impermeable. GSB conjugates were visualized by laser scanning microscopy and quantified by reversed-phase HPLC and fluorescence detection. Although in vitro labelling of plant extracts with monobromobimane (MBB) showed a threefold increase in total GSH after Cd treatment, the amount of GSH accessible to in situ labelling with MCB was significantly reduced in these cells compared to non-treated controls. This result suggests that in the presence of Cd a large fraction of the glutathione pool is not accessible to MCB anymore. Competition of MCB and Cd for free GSH may be interpreted as evidence for chelation of Cd by GSH. The fact that GSH is not increased in stoichiometric amounts compared to the accumulated Cd is explained by a high turnover of $Cd[GS]_2$ in the vacuole. High degradation activity for glutathione conjugates in the vacuole is shown to result in accumulation of the respective γ-EC- and cystein conjugates.

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Impact of tropospheric Ozone on Food and Feed Quality of Brassica species (OFFQ)

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Tropospheric ozone is the third most important greenhouse gas and its concentration is still increasing. This will have detrimental effects on plant productivity and cause changes in yield quality of agricultural and horticultural crops. These effects are primarily induced by an increased production of reactive oxygen species, which is a common feature of biotic (pathogens, insects) and edaphic stresses (drought, high light, UV, cold…). The "oxidative burst" activates signal transduction pathways that influence plant defence responses and the production of secondary metabolites such as vitamins and natural toxins e.g. S-containing glucosinolates. Glucosinolates, that are found exclusively in plants of the family *Brassicaceae,* have been attributed anticarcinogenic properties, whereas for animal feed they decrease the digestability and may cause e.g. goitre and haemolytic anaemia.
The presentation will focus on the influence of ozone on changes in metabolism of vitamins (ascorbic acid = vit C & α-tocopherol = vit E) and glucosinolates in oilseed rape (*Brassica napus* L.) and broccoli (*Brassica oleracea* L. cv. Italica). The experimental set-up consists of short term, controlled ozone exposure experiments in closed chambers, as well as more field related, season long ozone exposure in Open-Top Chambers. An effort is made to elucidate the interaction between abiotic stress induction, defence signalling pathways and changes in secondary metabolites by transcriptomic analyses. Therefore physiological assessments of plant stress responses (gas exchange and chlorophyll a fluorescence) are linked to biochemical analysis of antioxidants and glucosinolates as well as changes in gene expression at the leaf level. These aspects will be discussed based on the first year's results of this 4 year research project funded by the Belgian Science Policy Office.
The project aims to increase our understanding on the indirect influence of the environment on safety and health aspects of the food chain. Increasing knowledge of the plant-environment interactions will surely provide novel strategies to stabilise agricultural yield and quality in a fluctuating environment. This information is also imperative to be able to detect, monitor and understand the full impact of our changing environment, in order to identify the risks and justify the appropriate actions.

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Significance of Copper for the Uptake and Detoxification of Atrazine by Poplar Tree Species

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In many polluted soils, both heavy metals as well as organic pollutants such as pesticides are found in phytotoxic concentrations [1]. Thus, the performance of plants growing on such soils highly depends on their capability to detoxify both types of pollutants. Recent laboratory studies suggest that the presence of heavy metals can improve the detoxification of pesticides that are conjugated with the tripeptide glutathione (GSH) by nucleophilic addition reactions catalyzed by the glutathione *S*-transferase (GST) family. This improved detoxification has been attributed to a dual role of the enzyme phytochelatin synthetase (PCS), catalyzing the synthesis of heavy metal chelating peptides (phytochelatins) and being involved in the degradation of GSH conjugates [2]. Alternatively, pesticides such as atrazine may form complexes with heavy metals [3] that may be taken up and detoxified at a higher rate compared to its constituents [4]. The present study was performed to test these assumptions in a glasshouse experiment. For this purpose, cuttings of poplar tree species were exposed to soil containing copper and ^{15}N-atazine either alone or in combination. In the soil, removal of copper and ^{15}N-label were determined; in the plants, accumulation and distribution of copper and ^{15}N-label were analyzed. Chlorophyll fluorescence was taken as means of atrazine action, GSH levels, *GST* and *PCS* expression as means of detoxification of the pollutants. The significance of the results for the use of poplar for phytoremediation is discussed.

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INVITED TALK
Higher levels of lysine, threonine or cysteine affect the level of methionine in higher plants

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Lysine, threonine and methionine are three essential amino acids whose levels limit the nutritional quality of cereals and legume plants. These amino acids synthesized through the aspartate family biosynthesis pathway, in which lysine was produced through a different branch of threonine and methionine. To elucidate the relationship between these biosynthetic branches and to study the factors that regulate methionine synthesis, we crossed between transgenic tobacco plants overexpressing the Arabidopsis cystathionine γ-synthase (AtCGS), the first unique enzyme of methionine biosynthesis, which exhibits higher levels of methionine, and two different lines. The first line overexpressed feedback-insensitive bacterial enzyme dihydrodipicolinate synthase (bDHPS) that contains a significantly higher level of lysine, and the second line overexpressed the feedback-insensitive bacterial enzyme aspartate kinase (bAK). The results of the analysis of the progenies of plants expressing bDHPS/AtCGS together with analysis of feeding plants demonstrated that lysine reduced the expression level of *S*-adenosylmethionine (SAM) synthase, and as a result the level of SAM decreased, which led to a higher expression level of AtCGS and an increase in the level of methionine. Testing the second set of crosses (AtCGS/bAK), we next found that plants co-expressing both foreign genes have significantly higher methionine and threonine levels compared to levels found in wild-type plants. However, the methionine level does not increase beyond that found in plants expressing the AtCGS alone. This finding can be explained through the feedback inhibition regulation mediated by SAM on the expression level of AtCGS. To test this assumption, plants expressing bAK were crossed with plants expressing the AtCGS versions in which the domains responsible for the feedback regulation have been deleted. Indeed, significantly higher methionine levels accumulated in the newly produced plants. The results of this study indicate that the flux of the carbon/amino skeleton limits methionine synthesis. Next, we examined whether the level of cysteine limits methionine content. To test this, plants expressing the yeast *O*-acetylserine(thiol)lyase (OASTL) in the cytosol or in chloroplasts having a higher level of cysteine were crossed with those expressing the CGS. A slight but significantly higher level of methionine was found in plants expressing the plastic form of OASTL and CGS. However, in these plants, the level of glutathione significantly decreased and the plants were much more sensitive to oxidative stress. Plants expressing the cytosolic form of OASTL and CGS have higher levels of methionine and cysteine. The results of these studies suggest new ways of producing transgenic crop plants containing increased methionine levels, as well as higher methionine content together with threonine, lysine or cysteine levels, consequently having improved nutritional quality.

Regulation of uptake and distribution of sulfate in *Brassica*

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The sulfate uptake capacity and expression of sulfate transporters in *Brassica* were modulated to such an extent that its characteristic high sulfate content in the shoot was maintained even at sulfate concentrations as low as 5, 10 or 25 μM in the root environment. There was a substantial increase in the sulfate transporter capacity at ≤ 10 μM sulfate, which was accompanied with an enhanced expression of the apparent constituent sulfate transporters Sultr1;2 and 4;1 (maximal up to 4-fold). The Sultr1;1 and 4;2 sulfate transporters were hardly expressed under sulfate-sufficient conditions, though they were highly induced upon sulfate-deprivation (up to 35-fold). Prolonged deprivation resulted in an altered shoot to root biomass allocation in favor of that of the root. The transfer of sulfate-deprived plants to sulfate-sufficient conditions did not rapidly affect both the increased sulfate uptake capacity and the expression of sulfate transporters. There was a poor shoot to root signaling in the regulation of sulfate uptake capacity and expression of sulfate transporters upon sulfate deprivation. It was evident that the sulfate uptake by the root, but not the level of expression of the sulfate transporters, was strongly dependent on the shoot sink capacity for sulfate. The signal transduction pathway in the regulation of the uptake of sulfate and expression of the sulfate transporters will be evaluated.

The sulfate transporter gene family in wheat – is it different compared to Arabidopsis?

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For *Triticum* species such as wheat, the sulfate transporter (ST) family appears to be composed of only 10 genes in comparison to the 12 genes found Arabidopsis and rice. The high affinity subgroup contains 3 genes, however the 1.2 type is missing and a second 1.1 type resulting by gene duplication is present. The expression of the second 1.1 type is not influenced by the plant sulfate status, in contrast to the 1.2 type found in Arabidopsis or other plant species [1]. Genome analysis of *Brachypodium* indicated only one Group 2 ST. Genome and cDNA analysis revealed that the Group 2 of wheat also contains only one ST gene. In rice alone, 6 genes occur in Group 3, compared to wheat, *Brachypodium* and Arabidopsis, which all possess 5 Group 3 ST genes. Both wheat and rice have only one Group 4 gene in contrast to known dicotyledonous species, which have 2 Group 4 genes. As for Arabidopsis, sulfur nutrition affects expression of Group 1, 2 and 4 sulfate transporters. For example, in roots the expression of the high affinity TaSultr1;1a is strongly up-regulated under sulfur-limiting conditions. In contrast to Arabidopsis, an up-regulation of the TaSultr1;1a is also found in the shoots, which indicates a function of this ST in the high affinity cellular uptake of sulfate in shoot tissues when sulfate is limiting. In contrast to Arabidopsis, TaSultr1;3 is not influenced by the sulfate status of the wheat plant with strong expression in the shoots and weak expression in roots. The Group 2 ST is slightly up-regulated under sulfate limiting conditions in roots and shoots. Strong regulation of expression related to the sulfur status is found for Group 4 STs of wheat. A rapid and substantial increase of transcript abundance is visible within 24 h in roots as well as in shoots, in response to sulfate limitation, indicating the importance of sulfate release from the vacuole to maintain intracellular sulfate levels for metabolism and transport. The Group 3 STs are not influenced by the sulfur status of the wheat plant. Spatial and temporal expression patterns of selected wheat STs in seedling roots and shoots, and in mature plants during grain filling, in relation to sulfur availability will be presented and discussed.

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Distinct differences of two sulfate transporter from *Populus tremula* x *P. alba* that are expressed in phloem tissues

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Sulfur is one of the six macronutrients that is required in its reduced form for protein synthesis and, therefore, for growth and development. Sulfur is available to plants in the soil as sulfate that is taken up by the roots and then distributed within the plant by xylem and phloem transport. In the cells sulfate can be stored in the vacuole, transported into plastids for sulfate assimilation or it can be transported out of the cell to other plant tissues. The perennial growth pattern of deciduous trees requires special features not relevant in annual plants like *Arabidopsis* or rice. These features include storage and re-mobilization of nutrients in storage tissues of the trunk during the seasonal growth.
A total of 18 putative sulfate transporters that may be involved in uptake and distribution of sulfate within the plant have been cloned from *Populus*. Sequence comparison and phylogenetic analysis of the partial protein sequences indicate that these transporters cluster to the known five groups of *Arabidopsis* and other species. Northern analyses showed that two of these sulfate transporters are expressed specifically in bark tissues and are good candidates for phloem specific transporters which may be involved in phloem loading and/or unloading. *In situ* hybridization revealed that these two transporters indeed are expressed in phloem cells but show distinct differences. Their possible functions in phloem loading and/or unloading for storage of sulfate in bark parenchyma and ray pith cells as well as its contribution in whole plant sulfate cycling will be discussed.

Sulfate transporters in Vitis: roles and expression

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Sulfate is acquired from the soil by plant roots through a proton-sulfate co-transport system mediated by permeases displaying low Km. The active transport requires energy to overcome the strong electrochemical gradient trough the plasma membrane, considering that the soil solution is usually poor in sulfate. Several sulfate transporters with specific localizations are responsible for the initial uptake and distribution of sulfate throughout the plant. The sulfate transporter gene family can be aligned into 5 groups based on the predicted protein sequences [1]. Two sulfate transporters cloned from *Vitis vinifera* (*VvST* - EF155630) and *Vitis rupestris* (*VrST* - EF155629) clustered into group 1 which comprises genes for high-affinity sulfate transporters, regulated by S external conditions. After the recent sequencing of the *Vitis vinifera* genome [2,3] was possible the identification of various nucleotide sequences associated with sulfate transporters, a phylogenetic analysis showed that these sequences can be assorted into three of the five sulfate transporter family groups. A cell system of the two *Vitis* species and plantlets from *Vitis vinifera* were used to study the response to sulfate deficiency for 1, 3, 5 and 7 days, sulfate re-supply and the effect of several metabolites added to the medium (such as, *O*-acetylserine, *N*-acetylserine, cysteine, glutathione). Sulfate influx and the expression of different isoforms of sulfate transporters were evaluated, the influx by $^{35}SO_4^{2-}$ radioassay and gene expression by real-time PCR analysis. The effect of S starvation was impressive on the cell systems considering both aspects studied: after 24h in –S medium either $^{35}SO_4^{2-}$ influx or *VvST* and *VrST* expression were significantly higher when compared with cells exposed to full S medium. A clear result was observed in *Vitis vinifera* plantlets only after seven days without S. The re-supply of S to the medium led to a rapid down-regulation of the effect observed in –S conditions, although distinct in both species studied. Furthermore, the sulfate transporters from different groups showed distinct expression patterns in response to the culture conditions and in the different tissues. We will discuss the expression pattern observed for the sulfate transporters in different groups which probably confers distinctive roles during *Vitis vinifera* development and in t response to nutritional environment.

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Examination of the role of sulfate transporters expressed in seeds by using Arabidopsis T-DNA mutants

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Sulfur is an essential macronutrient needed for the synthesis of many cellular components (e.g. glutathione, flavonoids, and glucosinolates). In particular, sulfur is required for the biosynthesis of cysteine and methionine, which are important determinants of nutritional seed quality. By using the model legume *Medicago truncatula*, we have examined the distribution of transcripts for enzymes of sulfur assimilation in the three major seed tissues, seed coat, endosperm, and embryo, at the onset of seed filling. An intertissue compartmentalisation was revealed that may regulate the availability of sulfur for cysteine and methionine synthesis within the embryo [1]. These data, along with those from Tabe and Droux [2] demonstrated the seed's capacity to reduce sulfate in order to cope with storage protein synthesis. Understanding sulfate transport in developing seeds is of great interest since seeds of many crops, and particularly those of grain legumes, have a low sulfur amino acid content. In Arabidopsis, we identified seven genes encoding sulfate transporters expressed during seed development. Three of them belong to the group 3 sulfate transporters whose function remains to be elucidated. In order to examine the role of this sulfate transporter group, we undertook the characterization of Arabidopsis plants carrying a t-DNA insert in these genes. The effect of gene disruption on plant phenotype was evaluated, under limiting and non-limiting nutrient availability. Seed sulfate content, seed weight, germination, and the accumulation of the seed protein fractions were determined. The effects of the mutations allowed us to identify sulfate transporter genes implied in the determination of seed weight, yield, and protein composition. These results provide a basis for understanding the role of sulfate transporters in seeds.

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Characterization of the ATP Sulfurylase Gene Family in *Arabidopsis thaliana*

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The initial step of sulfur assimilation is the conversion of sulfate into adenosine 5' phosphosulfate (APS), an activation reaction catalyzed by ATP sulfurylase (ATPS) in the presence of ATP. Understanding the regulation of ATPS is important as this enzyme provides the entry point of sulfur into the assimilation pathway. The *Arabidopsis thaliana* genome encodes a family of four highly similar ATPS genes. Currently little is known about the full range of functions and regulatory mechanisms of the four protein isoforms that these genes encode, and differences between them remain to be elucidated. In some plant species, such as potato and spinach, two clearly distinct isoforms exist; one plastidic and one cytosolic. This compartmentalization implies independent functions, however, in Arabidopsis the isoform responsible for cytosolic ATPS activity is not known as all four genes encode putative chloroplast target peptides. I am looking to characterize the individual Arabidopsis ATPS isoforms using bioinformatics, in vitro biochemical analysis, expression profiling, reverse genetics approaches and molecular techniques. Information gained will help to generate a more complete picture of the roles and mechanisms of ATPS in higher plants.

Characterisation of the APS kinase gene family in *Arabidopsis thaliana*

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Upon assimilation in higher plants, sulfur is partitioned into components of primary and secondary metabolism. The branching point is the metabolism of adenosine 5'phosphosulfate (APS). APS can be reduced to sulfite by APS reductase, and after further reduction incorporated into cysteine and other components of primary metabolism, or phosphorylated by APS kinase (APK) to form PAPS. PAPS is the sulfate donor for sulfotransferase enzymes which catalyse the transfer of the sulfate group onto free hydroxyl groups of acceptor molecules. Such sulfated secondary metabolites include the glucosinolates and sulfated hormones, which play important roles in defence against biotic and abiotic stress. How the partitioning of sulfur between primary and secondary metabolism is controlled in plants is poorly understood. By investigating the APK gene family in Arabidopsis we hope to gain insight into the mechanism and control of sulfate partitioning. There are four isoforms of APK in Arabidopsis, which we are characterising using a combination of tools including reverse genetics, web-based and SQRT-PCR expression analysis, promoter::GUS / GFP reporter lines, biochemical analysis and metabolic profiling. Different expression patterns and subcellular localisation suggests distinct roles for individual isoforms within the family. Characterisation of single and multiple knockout mutants of APK is confirming this, with particular attention being paid to the glucosinolate profiles of the double mutants.

The up-regulation of *Vitis vinifera* sulfur assimilation by sulfate depletion decreases from cells to roots and to leaves

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Plants are able to assimilate sulfate (SO_4^{2-}) through three main metabolic steps: 1) activation with binding to ATP catalysed by ATP sulfurylase and forming adenosine phospho-sulfate (APS); 2) reduction to S^{2-} by the activities of GSH-APS reductase and Fdx-sulfite reductase; 3) incorporation of S^{2-} into cysteine (cys) by the activity of the complex serine acetyl transferase (SAT) and *O*-acetyl–L-serine (OASTL). By removing S supply some enzymes of the assimilatory pathway have their activities or mRNA pools increased [1] after several days in whole plants or several hours in cell suspensions. GSH-APS reductase gene seems to be the prime regulation point of the assimilatory pathway [2]. Although copper sulfate or elemental S are applied as fungicides to grapevine, no recent studies on SO_4^{2-} assimilation or effects of sulfur deficiency are reported for this species. Cell suspensions of two *Vitis* species, *Vitis vinifera* and *Vitis rupestris* and roots and leaves of *in vitro V. vinifera* plantlets were selected as experimental systems. Cell suspensions and *in vitro* plantlets grown under S-sufficient (+S) and S-deficient (-S) conditions, were used to study the regulation of the main sulfate metabolism enzymes and correspondent genes by SO_4^{2-} deficiency, re-supply and added metabolites (e.g., GSH, cys, OAS, NAS). Partial sequences of genes coding for APS reductase (EU275236) , SAT (EU27523) and OASTL (EU275237) were cloned and its expression was analyzed under +S and -S conditions in grapevine cells, roots and leaves by real-time PCR. Subsequently to grapevine genome sequencing [3], [4], we found different putative isoforms which respond differently to sulfate depletion. Searching for the influence of -S on plantlet branching we found that genes for some proteins of cytokinin transduction signal [5], [6], were clearly affected. The regulation of the expression of those genes by sulfate availability will be discussed.

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S resupply to S-deficient barley plants allows restoration of their capability to cope with Fe shortage

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The effect of the S nutritional status on plant capability to cope with Fe shortage was studied in solution cultivation experiment in barley (*Hordeum vulgare* L. cv. Europe). Barley is a Strategy II plant and responds to Fe deficiency by secretion of chelating compounds, phytosiderophores (PS). All PS are derived from nicotianamine whose precursor are methionine and S-adenosylmethionine. This finding reasonably suggests that a long-term supply of inadequate amount of S could reduce plant capability to respond to Fe deficiency by limiting the rate of PS biosynthesis.

We investigated the responses of barley plants grown for 12 d on Fe-free nutrient solutions (NS) containing 0 or 1.2 mM SO_4^{-2}, followed by a transfer to NS containing 1.2 mM SO_4^{-2} for time periods varying from 24 to 48 h.

Transferring the S- and Fe-deficient plants to S-sufficient NS did not significantly affect the growth rate of plants or leaf chlorosis. However, after the supply of S was restored to S-deprived plants, increase in PS release in root exudates was evident just after 24 h of growth in S-sufficient NS and the increment reached values up to 4-fold higher than the S-deficient control after 48 h from S-resupply. When S was supplied to S-deficient plants, leaf ATPS and OASTL activities exhibited a progressive recovery, most likely suggestive of an increase of S assimilation rate in leaves. It seems, however, that the response of barley plants to S resupply does not involve enhanced assimilation of sulfate at root level since the activity of two enzymes of assimilatory pathway (ATPS and OASTL) did not increase. Furthermore, root *HvST1* (high affinity sulfate transporter) transcript abundance remained elevated for 48h following S resupply and we also found a significant increase in the level of root *HvYS1* (Fe-PS transporter) transcripts after only 24 h of S resupply.

Data support the idea that the extent to which the plant is able to cope with Fe starvation is strongly associated with its S nutritional status. In particular, our results are indicative that barley plants fully recover their capability to cope with Fe shortage after the supply of S was restored to S-deficient plants.

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INVITED TALK
The impact of dietary sulfur amino acid intake on immune functions in health and disease.

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The sulfur amino acids (SAA) & metabolites are important in health & disease processes. Methionine is nutritionally essential. Cysteine, although synthesised from methionine, is semi-essential due the variable capacity of the body to produce it from methionine. The metabolic pathway requires folic acid, vitamin B6 and B12 as co-factors & is influenced by genetics [1]. Immune system activation, involves a complex interrelated series of cellular & metabolic activities. There are 3 components of the response - a non-specific inflammatory response, driven by pro-inflammatory cytokines, activation of T lymphocytes to attack the invading pathogen & production of antibodies which bind to the pathogen and aid its destruction. The cytokines –interleukin-1 (IL-1), IL-6 & tumor necrosis factor-α cause profound metabolic changes in the body. These a)create a hostile environment for the pathogen (raised body temperature, oxidant production and tissue inflammation, b)release of nutrients from within the body for 'feeding' the immune system, and c)initiate healing[2]. Paradoxically an excessive inflammatory response is deleterious and underlies the pathology that leads to increased morbidity & mortality following infection. High levels of inflammation also suppress T cell function. Also, oxidants, produced during the response, enhance cytokine production thereby boosting the inflammatory response. The effect is due to the activation nuclear factor-kappa B (NFkB), a nuclear 'switch' which turns on transcription of the genes responsible for the inflammatory process. An adequate or raised intake of sulfur amino acids prevents this situation [3].

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Session III: **Evolution of the pathway, variety of S metabolites and their significance**

Sulfur metabolism in marine diatoms

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Diatoms are eukaryotic, photosynthetic microorganisms found throughout marine and freshwater ecosystems and are responsible for as much as 20% of global primary productivity. Marine diatom *Thalassiosira pseudonana*, a model in this study, lives in sulfur-rich environment, where its growth is not limited by this element. Therefore we use diatoms to describe metabolic flow of nutrients and their relation to 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, recent sequencing 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.

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Sulfur metabolism in fungi: pathways and regulation

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Fungi, like plants, possess sulfate assimilation pathway. Its final product is sulfide which is incorporated into serine or homoserine carbon chains generating cysteine or homocysteine, respectively, depending on the organization of sulfur amino acid biosynthetic pathways in a given fungus. Three types of this organization, represented by *S. cerevisiae, S. pombe* and *A. nidulans* have been described [1]. The latter fungus possesses alternative pathways of cysteine synthesis – both have to be impaired to get cysteine-requiring auxotroph.
The are several regulatory systems involved in the regulation of sulfur metabolism. The best known is the sulfur metabolite repression system (SMR) which controls expression of several sulfur-related genes, particularly those encoding sulfate assimilation pathway enzymes. The system consists of genes coding for components of the SCF-type ubiquitine ligase which inactivates the transcription factor specific for sulfur metabolism genes when cysteine is in excess [2]. In such condition sulfate assimilation is shut off.
We have also identified in *A.nidulans* a set of genes up-regulated by homocysteine which we call „homocysteine regulon"[3,4]. These are genes coding for enzymes directly or indirectly involved in homocysteine metabolism. An enhanced synthesis of these enzymes may be a mechanism ensuring removal of an excess of homocysteine which is toxic in higher concentration. It also acts as a stress factor inducing the unfolded protein response.

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Sulfate assimilation in lower plants and algae: Surprising lessons from sequenced genomes

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Sulfate assimilation is relatively well understood in flowering plants, but very little information exists on this pathway in lower plants and algae. Since the finding of a putative 3'-phosphoadenosine 5'-phosphosulfate (PAPS) reductase in *Physcomitrella patens*, an enigmatic enzyme thought to exist in fungi and some bacteria only, it has been evident that sulfur metabolism in lower plants may substantially differ from seed plant models. The genomic sequencing of two basal plant species, the Bryophyte *Physcomitrella patens*, and the Lycophyte *Selaginella moellendorffii*, and of several algal species including *Chlamydomonas reinhardtii*, *Thalassiosira pseudonana* or *Phaeodactylum tricornutum*, opens up the possibility to search for differences between lower and higher plants and algae at the genomic level. The genomes of these species contain a surprising number of new enzyme variants and fusion. Also the complexity of several gene families involved in sulfate assimilation is substantially different in the various genomes. The consequences for regulation of the pathway and evolution of sulfate assimilation in plants will be discussed.

Sulfite oxidase as a key enzyme for protecting plants against sulfur dioxide

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Recently we have demonstrated the existence of sulfite oxidase (SO) in plants [1]. It is a homodimeric enzyme containing molybdenum as catalytic metal. We have also solved the atomic structure of this enzyme [2]. Plant SO is a housekeeping enzyme present in all organs tested in *A. thaliana*. SO is localized to the peroxisomes [3] and we showed that it uses oxygen as final electron acceptor molecule thereby producing hydrogen peroxide [4]. Sulfur dioxide (SO_2) is known as strongly damaging air pollutant. After conversion to sulfite in aqueous solution it becomes a strong nucleophilic agent that attacks numerous compounds in the cell. Therefore, plants have developed mechanism to control sulfite levels. We will show that SO is essential for detoxifying excessive amounts of sulfite in the cell which is important for survival of the plant [5]. T-DNA tagged *Arabidopsis thaliana* plants lacking the enzyme showed a decrease in vitality during SO_2-fumigation and a change in their S-metabolites. The same was found with RNAi-plants that we have generated for tobacco. On the contrary, overexpression of SO helped the plant to survive SO_2 concentrations that are detrimental for non-transformed wildtype plants, as was shown with poplar plants which are known to be particularly sensitive to SO_2 gas. Fumigation induced expression of the enzyme as demonstrated by promoter-reporter gene fusion, by immuno-blot analysis of SO-protein and by induction of enzyme activity. This implies that SO, as an otherwise constitutively expressed protein, is under additional control by the environmental factor SO_2. Finally, we will speculate about the function of SO under ambient conditions where SO_2 gas does not occur or is seen only very rarely [6].

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Reduced sulfur in the plant cell – enzymatic formation and functional roles

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Sulfur can be found in several oxidation states in the cell, either in the free form or as part of organic molecules. This paper describes exemplarily enzyme families involved in the formation of sulfur in different oxidation states and illustrates the diverse functions of sulfur containing molecules in the organism.

All members of the sulfotransferase (SOT, EC 2.8.2.-) protein family transfer the sulfur molecule in its highest oxidation state as sulfate to an appropriate hydroxyl group of several classes of substrates by using 3'-phosphoadenosine 5'-phosphosulfate as sulfuryl donor. In plants, sulfate conjugation reactions seems to play an important role in plant growth, development and adaptation to stress. The genome of *Arabidopsis thaliana* contains in total 21 genes that are likely to encode SOT proteins [1]. Many of their substrates, and therefore the respective physiological roles, of plant SOT proteins are not known. In *Arabidopsis* three SOT proteins catalyze the last step of glucosinolate (Gl) biosynthesis. *In vitro* enzyme assays revealed preferences of the recombinant SOT proteins for chemically different types of Gls. The putative role of SOT proteins in the manipulation of Gl biosynthesis and regulatory aspects will be discussed.

Sulfurtransferases (Str) comprise a group of enzymes widely distributed in archaea, eubacteria, and eukaryota which catalyze the transfer of a sulfur atom from suitable sulfur donors to nucleophilic sulfur acceptors. The best characterized Str is bovine rhodanese which catalyses *in vitro* the transfer of sulfane sulfur from thiosulfate to cyanide, leading to the formation of sulfite and thiocyanate. Str are differentially expressed in dependency on the nutritional status as shown by Northern Blot analyses [2]. To identify putative *in vivo* substrates a number of compounds have been tested in *in vitro* assays using several purified recombinant Str proteins in different kinds of enzymatic analyses. Conformational analysis done indicate the catalysis of larger molecules, such as proteins, as substrates for mitochondrial two-domain Str [3]. Biochemical data and bimolecular fluorescence complementation demonstrate *in vitro* and *in vivo* interaction with mitochondrial *Arabidopsis* thioredoxin, respectively.

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Identification of novel sulfur-containing metabolites bound to arabidopsis glutathione transferases

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Plant glutathione transferases form an abundant, diverse and highly stress-responsive family of proteins with well characterised roles in xenobiotic detoxification but with poorly understood endogenous functions. One such role is the binding and transport of reactive intermediates and to investigate this, a series of *in vitro* and *in vivo* screens have been employed. 51 of 52 transcribed GSTs in *Arabidopsis thaliana* were cloned; 49 were subsequently expressed as soluble enzymes using a custom Strep-tag system in *E. coli*. Metabolic screening of bacteria overexpressing these GSTs using reversed phase chromatography of methanolic extracts coupled to UV and electrospray mass spectrometric detection allowed the detection and identification of novel metabolites accumulating on expression of certain GSTs. GSTF2 and GSTF3 expression resulted in the accumulation of a number of heterocyclic aromatic compounds. This accumulation was due to GST binding, with sub-micromolar dissociation constants determined. Expression of GSTU7 and GSTU19 resulted in the accumulation of glutathione-conjugated porphyrinogens. The ligand-binding properties of GSTF2 and GSTU19 were examined further by overexpressing the Strep-tagged enzymes in tobacco and arabidopsis using custom binary vectors. Affinity-isolated protein was examined for bound ligands. Tobacco-expressed GSTF2 co-purified hydrophobic flavonoids and glutathione-conjugated lignanamides while arabidopsis-expressed GSTU19 co-purified a series of indole-glutathione polysulfides. Both enzymes also co-purified glutathione conjugates of the oxylipin OPDA. These results have significantly broadened the diversity of known GST ligands and the potential biological implications of this will be discussed.

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Metabolism of Sulfonated Aromatic Compounds in Plants

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In the development of a phytotreatment for effluents from dye and textile industry contaminated with sulfonated aromatic compounds, rhubarb and rumex, two plants producing natural anthraquinones, as well as non-producing plants like maize and celery, were tested for their ability to metabolise five sulfonated anthraquinones.
A previous study has shown that: 1) isolated rhubarb cells cultivated in bioreactors are able to accumulate and transform sulfonated aromatic compounds, and to desulfonate several of them [1]; 2) all plants tested, cultivated under hydroponic conditions, are able to take up and translocate sulfonated anthraquinones to the shoots, but rhubarb and related species are much more efficient to remove these xenobiotic compounds from model effluents than maize or celery [2]. Phytotransformation of these xenobiotics is also likely to occur in all species tested in vivo. To determine if sulfonated anthraquinones might be transformed by the classical detoxification pathway, enzymatic investigations have been performed. However, glutathione S-transferase from the leaves of tested species do not show any activity with the sulfonated anthraquinones under investigation.
On the basis of the results obtained, other enzymatic pathways, either classical detoxification or specific to anthraquinone producing plants, leading to the metabolism of sulfonated anthraquinones in the plants were also investigated. Plants were cultivated under hydroponic conditions in the presence or absence of sulfonated anthraquinones (0.2 mM each). In a first step, the activity of cytochrome P450 mono-oxygenases, key detoxification enzymes also involved in other biochemical processes, was measured in different parts (root/rhizome and leaf) of plants exposed or not to sulfonated anthraquinones. For the measurement of cytochromes P450 activity, a new method based on the fluorimetric detection of oxygen consumed during P450-catalysed reaction with NADPH and sulfonated anthraquinones as substrates was used. In a second step, the peroxidases activity was assayed spectrophotometrically at 470 nm by the guaiacole oxidation rate in the same plant parts exposed or not to the pollutants. Results obtained indicate that the activity of both enzymes increased in the presence of sulfonated anthraquinones and were involved in their detoxification mechanisms.

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Effects of modified cysteine contents on subcellular glutathione metabolism

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Cysteine, the initial product of sulfate assimilation, is supposed to be the rate-limiting factor for glutathione (γ-glutamyl-cysteinyl-glycine) synthesis in non-stressed plants. Glutathione is an important antioxidant in plants and plays key (protective) roles in cell metabolism through activating defense genes, by sensing reactive oxygen species (ROS) and by participating in ROS-signaling pathways. In plants glutathione synthesis is thought to take place in plastids and the cytosol whereas cysteine synthesis is carried out in plastids, mitochondria and the cytosol after the assimilation of sulfate to sulfide, which exclusively takes place in plastids. Considering the above described compartment specific pathways, the availability of cysteine, especially in plastids and/or cytosol is essential for glutathione synthesis. As the availability of glutathione can directly be linked to the plants ability to fight and sense oxidative stress the availability of inter- and intracellular glutathione and cysteine contents is an important measurement about the physiological condition of the plant especially under stress situations. In the present study, we present a method that allows the visualization of glutathione and one of its precursor cysteine in all cell compartments simultaneously in one experiment at a high level of resolution. This method is based on immunogold cytochemistry and computer-supported transmission electron microscopy. By applying this method on several different transgenic and non-transgenic plant species (*Arabidopsis thaliana, Cucurbita pepo* and *Beta vulgaris*) it was not only possible to demonstrate the specificity and accuracy of this method, but also to obtain thorough knowledge about the subcellular distribution of glutathione and cysteine in plants.

In the present study, these results are summarized and data is presented on how the treatment with the cysteine precursor L-2-oxothiazolidine-4-carboxylic acid (OTC) affects the subcellular distribution of glutathione and cysteine. Additionally, data is shown on experiments performed with glutathione deficient *Arabidopsis* mutants, which demonstrate that under circumstances of permanent glutathione depletion large amounts of cysteine accumulate within the cells.

In summary, the present study gives a detailed insight into the subcellular distribution of glutathione and cysteine in plants and allows speculation about the importance of these components in certain cell compartments during abiotic and biotic stress situations. Future perspectives on how the subcellular distribution of glutathione and cysteine correlates with the subcellular distribution of glutamate and glycine are discussed.

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Autoregulation of Glutathione Biosynthesis At Translational Level by Glutathione Itself

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The expression of γ-glutamylcysteine synthetase (γ-ECS), a key enzyme in glutathione (GSH) synthesis, is controlled at the transcriptional, translational, and posttranslational level. This multilevel regulatory mechanism allows plants to regulate GSH biosynthesis in response to the myriad of environmental stresses that are mitigated by GSH. Here we present evidence suggesting that the translation of *GSH1* mRNA is regulated by a specific binding of a protein factor(s) to the 5' untranslated region (5'UTR) of this mRNA, thus blocking the translation. The binding of this factor appears to be positively correlated to GSH/GSSG ratio, making it a redox-sensitive switch for translation control in response to oxidative stress. Therefore, GSH may regulate its own synthesis by restricting the protein level of γ-ECS. The RNA-binding complex was purified using affinity chromatography. The protein complex was subsequently resolved with SDS-PAGE and its individual components identified with MALDI-TOF. *In vitro* reconstitution and biochemically characterization of the complex will be discussed.

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Control of root growth by glutathione

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The low molecular weight thiol glutathione (GSH) plays an important role in response to various biotic and abiotic stresses and as a storage and transport form of reduced sulfur. In addition, a mutant in GSH biosynthetic enzyme, γ-glutamylcysteine synthetase has been identified as root meristemless mutant (rml1). Indeed, reduction of GSH content by buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcysteine synthetase, results in significant reduction of root growth. Surprisingly, however, similar reduction in root growth is observed as a result of addition of GSH. To identify processes involved in regulation of root growth by GSH we performed a genetic screen of EMS mutagenised population and identified mutants with roots insensitive to BSO. To complement characterisation of the mutants we utilised natural variation in response to BSO between Arabidopsis Col and Ler ecotypes to perform a QTL analysis. The study thus demonstrates a tight link between plant nutrition and root development.

INVITED TALK
Toward comprehensive understanding of regulatory network of sulfur metabolism

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It is well established that sulfur metabolisms are regulated in response to sulfur conditions in the environment. Sulfate uptake, translocation and metabolisms are all affected by the sulfur conditions. Understanding of this regulatory network is important both for basic- and application-oriented research. The regulatory mechanisms of sulfur-regulated gene expression have attracted a number of scientists and studied extensively.
Cis and *trans*-acting elements for the regulatory mechanisms of gene expression have been identified through molecular biological and molecular genetic approaches. Effects of disruption of genes responsible for sulfate uptake and assimilation have been described. Genetic analysis of Arabidopsis mutants defective in sulfur regulated-gene expression revealed possible interaction between sulfur and nitrogen assimilation pathways. Transcriptome and metabolome analysis further deepened our understandings.
In this talk, I would like to introduce recent progress in this important research area mostly focusing on the output from Japanese research groups.

Transcription factors relevant to auxin signalling coordinate broad-spectrum metabolic shifts including sulfur metabolism

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We have previously used a systems approach to follow the response behaviour of *Arabidopsis thaliana* plants upon sulfur limitation. The connectivity of genes, metabolites, and genes to metabolites led to causal relationships linking the stressor (low sulfate) with physiological endpoints [1-4]. The resulting scale-free network allowed us to identify potential transcriptional regulators of sulfur metabolism. Here, we selected three sulfur-starvation responsive transcription factors, IAA13, IAA28, and ARF-2 (ARF1-Binding Protein), all of which are related to auxin signalling, for further investigations. *IAA28* over-expressing and knock-down lines showed no major morphological changes, whereas *IAA13* and *ARF1-BP* over-expressing plants grew more slowly than the wild-type. We monitored steady-state metabolite levels and expression of pathway-relevant genes under normal and sulfate depleted conditions. For all lines changes in transcript and metabolite levels were observed, yet none of these changes could exclusively be linked to sulfur stress. Instead, up or down regulation of the transcription factors caused metabolic changes which in turn affected sulfur metabolism. Auxin relevant transcription factors are thus part of a complex response pattern to nutrient starvation that serve as coordinators of the metabolic shifts driving sulfur homeostasis rather then as direct effectors of the sulfate assimilation pathway. This study provides the first evidence ever presented that correlates auxin-related transcriptional regulators and primary plant metabolism.

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Omics-based identification of the genes involved in methionine-derived glucosinolate biosynthesis

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Understanding of plant metabolism as a system is essential for metabolic engineering aimed at the effective production of compounds useful to human life. By integrating transcriptomics and metabolomics, we elucidated global regulation of the transcriptome and metabolome of Arabidopsis under nutritional stress conditions such as sulfur deficiency [1], and presented a strategy to identify novel gene functions comprehensively [2, 3]. Coexpression analysis based on condition-specific (i.e., sulfur deficiency) transcriptome data and on publicly-available condition-independent transcriptome data enabled us to predict the genes involved in glucosinolates (GSLs) biosynthesis comprehensively. Predicted gene functions were confirmed by the analyses of knockout mutants and overexpression lines of the respective genes. We have reported so far the identification of the genes encoding sulfotransferases and Myb transcription factors, PMGs (Production of Methionine-derived Glucosinolate). In this presentation we will report the identification of novel genes involved in methionine-derived GSLs.

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Local and systemic response of sulfur starvation

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Plants have to monitor cellular levels of a number of compounds at different levels, e.g. time, concentration and tissue. To homeostatic imbalances the plant system has to respond quickly by transducing appropriated signals. Under sulfur starvation the uptake of sulfur is optimized by activation of a number of genes encoding proteins involved in sulfur uptake and/or reduction. It has been postulated that signals exist connecting the sulfur demand of the shoot with the activity of the uptake system of the roots (Lappartient *et al.* 1999). To investigate this aspect in more detail we have used the split root system to get knowledge about potential systemic and/or local signals under sulfur starvation. The expression level of several sulfur pathways genes has been measured for roots and shoots of +s/–s treated plants and at the sides of the splited root and the shoot after 5 days starvation. The obtained results indicate clearly that the expression of the uptake system in the root is strongly controlled by the local availability of sulfur. A systemic response to sulfur starvation can not be excluded however is unlikely due to the obtained data. Details will be discussed.

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Investigating function of the genes induced by a short-term sulfur deficit: pleiotropic effects of modification the *UP9* expression level

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Screening of the tobacco cDNA libraries with SSH method for genes regulated by short term sulfur starvation revealed, among others, cDNA corresponding to the previously uncharacterized gene named in our laboratory *UP9* [1]. This gene focused our attention because of a very high frequency of identified *UP9* cDNA fragments that reflected strong increase of its expression during sulfur deficit. The deduced UP9 protein consists of 117 amino acids and contains a potential nuclear localization site, coiled-coil structure and putative phosphorylation site. Study of *UP9* expression in stressed tobacco plants confirmed very strong regulation of this gene by sulfur starvation and suggested its specificity to sulfur starvation stress. To resolve the problem of UP9 function we used a multi-technique approach, including screening of databases, construction of lines with changed *UP9* level (*UP9* "sense" and "antysense" lines) and their phenotypic, biochemical, and molecular characterization. We have also identified the protein partners of UP9 with yeast two-hybrid system using libraries prepared from tobacco plants grown either in optimal or in S-deficient conditions. Despite variety of experiments the exact role of UP9 still remains unresolved, however observed pleiotropic effects suggest importance of this protein in plant metabolism, and encourage us for further investigation. The hypothesis for the possible functions of this protein will be discussed during the talk.

Acknowledgement: This work was supported by grant PBZ-KBN-110/P04/2004 from MNiSW.

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Using gene trap to develop plant bioindicators for sulfur nutritional status.

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The gene trapping strategy is a powerful tool to reveal functional aspects of essential genes along with their tissue specificity and inducibility. We are exploiting this strategy to identify plant S responsive genes, useful to develop plant bioindicators for monitoring plant S nutritional status and/or sulfate availability in the rhizosphere. To this purpose, we are screening a collection of Arabidopsis gene trapping lines generated by the "EXOTIC" consortium, by insertional mutagenesis with a modified maize *Ds* transposable element, carrying the promoter-less *GUS* gene as a reporter. In lines having *GUS* inserted within or near a chromosomal gene, *GUS* expression mimics that one of the chromosomal gene. In particular, we are searching for lines that show a differential expression of *GUS* in the presence or absence of sulfate, the main S source for plants. To date we have identified a line showing *GUS* expression in the root apexes and shoots only when grown under sulfate starvation. The growth of this line on media lacking in others nutrients (N, P, K, Mg, Ca, Fe), with different sulfate concentrations, or with different S sources (Cys or sulfate), showed the reporter activation to be specific for S withdrawal and dependent on the strictness of the imposed nutritional stress. The genomic sequences flanking the transposon insertion were identified as the intergenic region between *AT1G12030* (gene of unknown function, induced by S withdrawal) and *AT1G12040* (not responsive to S, encodes a leucine-rich repeat/extensin like protein). RT-PCR analyses showed that the expression of the flanking genes was not influenced by the transposon insertion, suggesting that the observed behaviours were due just to the presence or absence of sulfate in the growing medium and not to a general alteration of the transcriptional responses of the line. These results suggest that the identified intergenic region is able to induce the expression of a reporter gene under S starvation and it is thus exploitable for developing plant bioindicators of S nutritional status. The specific features of this region are probably due to the presence of cis-acting elements probably involved in the transcriptional control of *AT1G12030* under S starvation, and able to induce the gene activation in two different directions; *in silico* analyses identify the *SURE* element of *Sultr1;1* [1] as a good candidate.

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The remobilization of leaf N and S compounds of oilseed rape in response to sulfate deficiency depends on nitrate availability in soil

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Oilseed rape (*Brassica napus* L.) is a crop plant very demanding in both sulfate and nitrate which paradoxically exhibits a weak N remobilization leading to a low N use efficiency. For about twenty years, the decrease of the industrial rejections of sulfur (about -50% between 1980 and 2000 in France) results in a sulfate impoverishment of the soil. The changes of S and N metabolisms caused by the decline of sulfate availability in soil severely alter the agronomic performances of oilseed rape. Assuming that an efficient recycling of S and N compounds from senescing leaves to young growing tissues may improve the responses of oilseed rape to transient S deficiency, the effects of low sulfate availability provided for 35 days and combined or not with nitrate deficiency were investigated on S and N remobilization processes associated with leaf senescence. To study the effect of sulfate deficiency as function of nitrate availability on the senescence progression, an accurate molecular indicator of leaf senescence status (*SAG12*/*Cab*) was used [1]. Thus, in comparison with control High N-High S (HN-HS) plants, the Low N-Low S (LN-LS) treatment rapidly decreases the total leaf biomass and accelerates leaf senescence. In High N-Low S (HN-LS) plants, the growth of young leaves is significantly reduced only after 28 days and the senescence is delayed. In maturating leaf of LN-LS plants, the soluble protein amount rapidly decreases (since 14 days) while it remains similar between HN-HS and HN-LS plants. While the sulfate concentration remains at high level in maturating leaf of HN-HS plants, it decreases by 2 between 7 and 14 days for HN-LS plants and by 1.5 between 14 and 21 days for LN-LS plants. The high decline of sulfate concentration in maturing leaf of HN-LS and LN-LS was associated with an induction of the expression of *BnSultr4;1* (a gene encoding a vacuolar sulfate transporter suspected to be implicated in sulfate efflux). Overall data indicated that remobilization of S and N compounds, in response to sulfate deficiency, depends on nitrate availability. A better understanding of S and N recycling processes would change the way of adding chemical fertilizers to oilseed rape fields.

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Uptake, translocation and metabolism of selenium in *Arabidopsis thaliana*

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The usefulness of selenium as a tracer for the uptake, transport and metabolism of sulfur in plants was tested. Selenium containing compounds such as selenate and selenocysteine are chemical analogues of the corresponding sulfur compounds (e.g. sulfate and cysteine, respectively). In microbes, selenate is acquired and metabolised by the proteins of the sulfate assimilation pathway to produce seleno-cysteine, and this is probably also the case in plants. Because of the relatively high atomic mass of selenium, synchrotron-based K-edge X-ray absorption spectroscopy can be used to quantify selenium in biological samples and to determine the relative amounts of the different chemical species containing this element.

Many of the plant genes involved in transport and metabolism of sulfate have been identified. These include the 14 sulfate proton symporter genes and the four ATP-sulfurylase genes of *Arabidopsis*. However, the roles of specific genes in whole-plant physiology and the extent to which these roles are redundant are still far from being fully elucidated. We have used crossing and selection to accumulate T-DNA insertion, gene-knockout mutations in *Arabidopsis* lines. The finished lines carry mutations in most or all of the genes representing specific gene families with roles in either sulfate transport or sulfate metabolism. These *Arabidopsis* lines have been grown hydroponically with defined nutrient supplements to determine dry-matter accumulation. They have also been challenged with selenate to determine the rates of selenate uptake and transport, the sites of selenium accumulation and the extent and nature of selenium metabolism. The mutant phenotypes and their significance will be discussed.

Selenium is an essential micronutrient for humans and other animals. In addition to providing insights into plant sulfur metabolism, this research is also developing information that will facilitate the future development of plants with an improved ability to acquire environmental selenium and accumulate it in bio-available forms.

Regulation of Sulfolipid and Phospholipid Metabolism under Sulfur-starved Conditions in a Green Alga, *Chlamydomonas reinhardtii*

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Chloroplasts contain two acidic lipids, e.g. a sulfolipid, sulfoquinovosyl diacylglycerol (SQDG) and a phospholipid, phosphatidylglycerol (PG). When a green alga, *Chlamydomonas reinhardtii* was exposed to sulfur-starvation, the content of SQDG was decreased through the induction of degradation systems [1]. On the other hand, that of PG was increased up to a level that just compensates for the loss of SQDG through the induction of the PG synthesis. Similar activation was also observed in an SQDG-deficient mutant under S-replete conditions [2]. These results indicated that upregulation of PG synthesis under S-starved conditions occurs through the direct sensing of SQDG-loss, but not of S-starvation.

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Preferred session: II Sulfur metabolism and its interaction with other metabolic pathways

Cellular redox homoeostasis and gutathione reduction in *Arabidopsis thaliana*

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Glutathione is a major constituent of redox homeostasis in several compartments of the plant cell, including the organelles, cytosol and ER. To understand this function the redox state of glutathione, its exchange between compartments and control of biosynthesis need to be known, preferably in life cells. Arabidopsis T-DNA mutants were used in combination with a redox-sensitive GFP (roGFP) as a probe to analyse glutathione and redox homeostasis [1,2]. Exchange of glutathione between the compartments is implicated by the sole presence of glutathione reductase (GR) in plastids, mitochondria and cytosol. The membrane transport mechanisms are still largely elusive but may occur as reduced, oxidized, conjugated or by way of the glutathione cycle found in animals. To investigate the role of GRs in the three compartments, T-DNA insertion lines of the two *GR* genes of Arabidopsis were characterized. GR1 encodes plastid and mitochondrial GR by way of a dual target sequence. A null allele of *gr1* was embryo lethal. Inactivation of *gr2* encoding cytosolic GR2 caused no visible phenotype, but resulted in 60% reduction of overall GR activity. The cytosol had significantly increased contents of oxidized glutathione and consequently a lowered glutathione redox potential in the cytosol as shown by ratiometric fluorescence measurement of roGFP targeted to the cytosol of *gr2* plants. To dissect the role GR1 in organelle redox homeostasis the *gr1* mutant was complemented by plastid- or mitochondria-specific constructs. Expression of GR1 only in plastids was sufficient for survival while exclusive targeting to mitochondria was not. Thus, exchange of reduced or oxidized glutathione or of precursors across the plastid membrane was insufficient to maintain glutathione redox homeostasis inside the plastids. This demand for plastidic glutathione reduction capacity is already essential during early embryo development long before initiation of chlorophyll biosynthesis.

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Analysis of the O-acetylserine(thiol)lyase gene family demonstrates compartment-specific differences in the regulation of cysteine synthesis

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In plants, the last step of cysteine biosynthesis is catalyzed by the enzyme family of *O*-acetylserine(thiol)lyases (OAS-TLs) whose members locate to the cytosol, the plastids and the mitochondria. The reason for the presence of cysteine biosynthesis in each of these compartments may be limits of cysteine exchange between them or specific roles of the OAS-TLs in the individual compartments. Analysis of Arabidopsis T-DNA insertion lines for the different OAS-TLs revealed that cysteine is freely exchangeable between the three compartments. Rather, the proposal of specific roles for the individual OAS-TLs seems to apply. While OAS-TL A in the cytosol is responsible for the synthesis of the majority of cellular cysteine in the Columbia wildtype, the mitochondria, containing the in terms of quantity very minor form OAS-TL C, seem to play a much more important role for the regulation of whole-cell cysteine homeostasis [1]. This observation is supported by feeding experiments with radiolabeled substrates. How the interaction of enzymes of cysteine synthesis in mitochondria achieve this metabolic regulation is being investigated in this study.

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Roles of gene families of *O*-acetylserine thiol-lyase and serine acetyltransferase in Arabidopsis: Just redundancy or hidden secret?

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O-Acetylserine thiol-lyase (cysteine synthase: CSase) and serine acetyltransferase (SATase) are committed in the biosynthesis of cysteine in plant. CSase forms cysteine from hydrogen sulfide and *O*-acetylserine produced by the action of SATase.
In *Arabidopsis thaliana,* nine genomic sequences encode putative *β*-substituted alanine synthase (Bsas) proteins comprising cysteine synthase (CSase) [*O*-acetyl-serine (thiol) lyase] and *β*-cyanoalanine synthase (CASase). The physiological roles of these Bsas isoforms *in vivo* were investigated by the characterization of T-DNA insertion mutants [1]. Analyses of gene expression, activities of CSase and CASase, and levels of Cys and glutathione in the *bsas* mutants indicated that cytosolic Bsas1;1, plastidic Bsas2;1, and mitochondrial Bsas2;2 play major roles in Cys biosynthesis. Cytosolic Bsas1;1 has the most dominant contribution both in leaf and root, and mitochondrial Bsas2;2 plays a significant role in root. Mitochondrial Bsas3;1 is a genuine CASase. Nontargeted metabolome analyses of knockout mutants were carried out by a combination of GC-TOF and CE-TOF mass spectrometry. The level of □-glutamyl-□-cyanoalanine decreased in the mutant *bsas3;1*, indicating the crucial role of *Bsas3;1* in □-cyanoalanine metabolism *in vivo*.
To investigate the function of five SATase-like genes (*Serat*), we have isolated quadra-knockout mutants, in which only each single *Serat* gene remains, by crossing the each T-DNA mutant. Since all quadra-knockout mutants could grow albeit with varied extents and the quint-knockout mutant was embryogenically lethal, any *Serat* gene can support the growth of Arabidopsis and a plant cannot survive without *Serat* genes, suggesting no other pathway can replace with one by Serat for cysteine production.

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Monitoring of protein-protein interaction in the cysteine synthase complex in vivo

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Cysteine is a key molecule in the entire sulfur metabolism of plants. The rate of its synthesis is strictly controlled and coupled to the activity of assimilatory sulfate reduction and demand by downstream metabolic pathways such as glutathione and protein biosynthesis. It is catalysed by serine acetyltransferase and O-acetyserine (OAS) (thiol) lyase that form the hetero-oligomeric cysteine synthase (CS) complex in different subcellullar compartments [1]. In vitro studies showed that the CS complex can be stabilized by sulfide and dissociated by OAS, resulting in inverse activation/deactivation of the subunits. The regulatory model of the CS complex predicts a dual function as a sensor and trigger for the control of cellular sulfur homeostasis [2]. To demonstrate the reversible protein-protein interaction of the CS complex in vivo the subunits were fused with YFP and CFP. Simultaneous expression in tobacco protoplasts resulted in interaction of the CS complex subunits and concomitant fluorescence energy transfer (FRET) between CFP and YFP. FRET efficiencies were determined from acceptor bleaching and verified incomparison to controls and reverse directions of transfer between subunits. FRET was observed after targeting of fusion proteins to the cytosol and mitochondria. Moreover, feeding of protoplasts with either sulfide or OAS stabilized or eliminated FRET as predicted by the CS complex regulatory model and were corroborated by HPLC analysis of metabolite concentrations in the transfected protoplasts. The data strongly support the function of the CS complex in vivo as a dynamic sensor of intermediates of cysteine synthesis that result from the sulfur and nitrogen assimilation pathways.

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Posters

P1

National survey of S availability for field crops in Finland

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National S survey was carried out in Finland in summer 2006 to evaluate the status of S availability to the crop plants. Farmers provided plant samples of major field crops throughout Finland: barley and timothy samples from southern Finland up to latitude 65 ºN, while turnip rape and pea samples were gathered from their main production region in southern and south-western Finland. Leaf samples were collected just prior to heading (barley and timothy) or onset of flowering (turnip rape and pea) for malate-sulphate analysis carried out in Farm Hill Research, UK [1]. Along the plant sample, farmer provided some background information including: rural district, soil type, previous crop, use or non-use of compound fertilisers fortified with sulphur and whether conventional or organic farming was practiced on the farm. Critical malate-sulphate values indicating potential yield penalties were specified in control condition experiments for each crop species [2, 3]. The main result of the survey was that S availability seems to be at sufficient level to fulfil crops need in majority of the studied plant samples. In general, malate-sulphate values were low, but crop species differed in their mean values: highest value was recorded for pea followed by timothy, whereas barley and turnip rape produced clearly lowest mean values. Even though low values were recorded for turnip rape, it is considered to be likely the first crop species to suffer S deficiency. Geographical difference was evident as the regions in the northern part of the country produced lower estimate for malate-sulphate value. Also soil type had an influence, as organic soils produced lower values compared to clay and sandy soils. Use of S containing fertilisation resulted in lower values in clay and sandy soils. As a conclusion, in contrast to many European countries, common use of fertilisers containing S combined with lower national yield level results in largely sufficient S status in Finnish soils.

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P2

Study on the sulfur nutrition of the sugarcane and balance of sulfur in soil for sugarcane planting area

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Guangxi located in the southwest of China, it is to belong tropical to subtropical monsoon climate district, the temperature and rainfall are relatively high, the soil is weathered and leached the function of dissolving strongly, the phosphorus, potassium, sulfur and cation exchange capacity (CEC) are relatively low in the soil [1]. In recent years, because the improvement of sugarcane yield increased in a large amount and the reduction of the sulfur fertilizer applied on sugarcane. So, it is significant to promoting Guangxi sugarcane and producing sustainable and stable development to this experimental study.

The soil of sugarcane planting areas in Guangxi has larger areas that lack the sulfur and lack the sulfur potentially. OPT (+S) treatment increased sugarcane available stem of 8700 plant/hectare, or 12.1% and single stem weight of 120 grams, or 10.6% than the treatment without sulfur fertilizer. This is the fertile foundation of sugarcane yield. Application of 30 kg S/ha and 60 kg S/ha in the previous year, the treatments increased sugarcane yield of 2.49% and 7.31% than treatment without S. After the application of sulfur fertilizer on sugarcane, the cane sugar is divided and increased by 0.06%, the fibre is divided and improved by 0.17%, reduce candies to reduce by 0.05%. Each hectares of amount of sugarcane absorption sulfur reached 44.1-67 kg. Average yearly rainfall is 1379 mm., sulfur that rainwater brings 17.4 kg/ha. Because rainwater brings sulfur of SO_4^{-2}. Sugarcane absorbed sulfur only 30% of utilization ratio from rain, the annual rainwater brings sulfur of 5.22 kg/ha to use by sugarcane. From nutrient balance analysis, there were surplus of sulfur nutrient that OPT treatment applied sulfur fertilizer, and the treatment without application sulfur fertilizer, sulfur nutrient lose 23.67 kg/ha for one year because sugarcane stem uptake sulfur nutrient from soil.

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P3

Effect of S-deficiency on the genetic control of nutrient remobilisation during wheat (*Triticum* aestivum L.) grain-filling

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Wheat is the major arable crop grown in the UK with 15 million tonnes produced annually supplying 85% of the country's requirement for milling, bread making and animal feeds. Grain yield and quality depends on nutrient availability throughout the development of the crop and on the internal remobilisation of nutrient resources to the seed during grain development.

Sulfur (S) and nitrogen (N) are critical to the breadmaking quality of wheat. S-nutrition is specifically associated with levels of glutenin in the endosperm and the ratio of glutenin to other grain storage proteins. These factors are responsible for dough elasticity and loaf quality [1, 2]. During growth of the wheat crop, S is accumulated in the vegetative tissues and is redistributed to the developing seed in both organic and inorganic forms.

We have studied the remobilisation of S and N from field-grown wheat leaves during grain-filling. Differing strategies were observed for the export of these two major nutritional elements from senescing leaves. Using Rothamsted's Broadbalk experiment, physiological, transctriptomic (Affymetrix) and metabolomic analyses were carried out to analyse the molecular basis of nutrient remobilisation and the effect fertiliser deficiencies have on the key processes. Major effects of S-deficiency on S and N economy at the crop and genetic and level will be presented.

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P4

Replacement of different amounts of sulfur and *Thiobacillus* inoculant on wheat yield and quality

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For yield increase in hectare beside of using of up- yield varieties, carrying out of other agronomic actions such as optimum using of fertilizers and water is necessary. Due to positive effects of sulfur on soil and growth of plant and because of low oxidation of sulfur in soil, this study was carried out to experim the effects of different amounts of sulfur and inoculant of *Thiobacillus* bacteria on wheat. The experiment was factorial in completely block design with 2 factors in 3 replications. One factor was different amounts of powdered sulfur (s) 0, 200, 400 and 800 kg ha^{-1} and another factor was different amounts of inoculant 0, 0.5 and 1.0 weight percent of used sulfur. An additional treatment (treat 13) was used *Thiobacillus* on 0.5 weight percent for 200 $kgha^{-1}$ S without using of S. Factors of concentration of elements in wheat leaf, grain and shoot, grain yield and shoot and amounts of elements in soil after harvest was determined. Results were analyzed with S A S program (9.1 V) and Duncan test. Based on results, main effects of sulfur on N and Zn concentration of leaf was statistically significant but effects on *P*, Fe, Cu and Mn wasn't significant. Main effect's of inoculant on leaf Zn and Mn was significant. Interaction of two factors on Fe, P. Cu and Mn concentration was insignificant and on Nitrogen and Zn was significant. Interaction of two factors on K, Cu and Mn of shoot was insignificant and on P, S. Fe and Zn of shoot was significant. Effects of S was only on S concentration of grain significant. Effects of inoculate on grain concentration of S, Zn and Cu was significant. Interaction effect of two factors on N, P and Fe concentration of grain was insignificant and on k, S, Zn, Cu and Mn were significant. Main effects of S or inoculant on total, shoot and grain yield and leaf chlorophyll index was insignificant and interaction of two factors on grain yield and leaf chlorophyll index was insignificant and on total and shoot yield was significant. Main effects of two factors on N, P, Zn and S uptake of shoot was significant and on Fe, K, Cu and Mn uptake of shoot was insignificant .Main effect of inoculants was significant on S uptake of shoot. Main effects of different amounts of sulfur only on S uptake of grain was significant. Between three amounts of sulfur there was no significant difference. Main effects of inoculant on grain S, Zn and Cu uptake was significant and in thio 1.0 was further. Interaction effects of two factors on grain N, P, Mn, Fe and Cu uptake was insignificant and on S and Zn uptake was significant. Furthest S uptake was belong to Thio1.0 S800 and furthest Zn and Mn uptake in grain was belong to Thio0.0 S200. Main effect of sulfur on N, S and Mn uptake of upper part (grain + shoot) was significant and Main effect of inoculants on Zn and Cu uptake of upper part was significant. Interaction effects of two factor was significant on uptake of nutrients in upper part unless in P. Based on results if so the main or interaction effects of two factors on grain yield were insignificant (perhaps it was sufficient amount of s in soil+ water) it had significant effects on nutrient uptake.

P5

The influence of fertilizers rich in elementary sulfur or sulfate on cadmium accumulation in potatoes (*Solanum tuberosum*)

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The plants need well-balanced macroelements as well as microelements uptake for their growth and development. Sulfur belongs to group of macroelements. This element and its compounds play many essential and crucial roles including detoxification of xenobiotics in plants [1]. The crops are supplemented by sulfur in the form of elementary sulfur or as ammonium sulfate.
Cadmium belongs to the group of so-called toxic heavy metals that are very harmful for all organisms even in very low doses. The main toxic effect of cadmium(II) ions bases in physico-chemical similarities with zinc(II) ions.
Tubers of *Solanum tuberosum* were cultivated in pots with the gley soil containing less than 0,001 $mg \cdot kg^{-1}$ of cadmium. At the end of the cultivation the plants were transplanted to the soil containing elementary or sulfate form of sulfur. The sulfur doses were 0, 20, 40 or 60 $mg \cdot kg^{-1}$.
The increasing content of elementary sulfur lowered cadmium accumulation in potato tubers (measured in dry mass) compared to control. Particularly in control plants the cadmium concentration was determined as 0.8 $mg \cdot kg^{-1}$, however the experimental group fertilized with 60 mg of elementary sulfur per kg of soil contained 0.5 mg of cadmium per kg of dry mass of the plants. In of the case of ammonium sulfate fertilizing (concentrations 20 and 40 $mg \cdot kg^{-1}$) the content of cadmium determined in tubers was lower compared to control potato plants. In contrary to all other experimental groups the group of plants fertilized by ammonium sulfate (60 $mg \cdot kg^{-1}$) contained the similar amounts of cadmium as control plants. Moreover the content of cadmium in above ground plant parts (as dry mass) was higher at all experimental groups compared to control plants. In addition we were interested in total content of thiol compounds in the whole plants. Their concentrations were increased up to eight day of the cultivation in the soil rich in both forms of sulfur. During eight and sixteenth day of the cultivation the decrease in amount thiol compounds was determined. This decrease was observable also after sixteenth day of the cultivation, but it was not so marked.

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P6

Effect of sulphur supply on amino acid accumulation in wheat grain and its implications for acrylamide formation during processing

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The precursors for acrylamide formation in wheat products are free asparagine and sugars which react in the Maillard reaction during high-temperature processing [1]. The main limiting factor in wheat flour is asparagine [2, 3]. Asparagine is produced mainly from glutamine and aspartate and accumulates in response to a variety of stresses including deficiencies in minerals, of which sulphur is the most important [4].

The effect of sulphur deprivation on amino acid accumulation in wheat grain was evaluated using double haploid lines derived from crosses between varieties Spark and Rialto. Amino acid levels were determined by gas chromatography – mass spectrometry (GC-MS). Double haploid lines grown under sulphur deficient conditions accumulated from 10- to 120-fold higher amounts of certain amino acids in their mature grain in comparison to the grain of the same lines grown under sulphur sufficiency. These results correlated well with those obtained by Muttucumaru and co-workers using different wheat varieties [2]. Analyses of variance revealed that the free amino acids could be divided into three groups on the basis of genetic (G), environmental (E) and G x E effects of sulphur treatment on their accumulation in the mature grain. The main conclusion to be drawn from this data is that levels of some amino acids, including asparagine, are controlled not only by environmental but also by genetic factors.

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P7

THE EFFECT OF SULFUR AND NITROGEN ON YIELD OF WINTER RAPE SEED AND ITS QUALITY

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The aim of presented researches was to test some sulfur doses and differentiated nitrogen supply in aspect of their influence on formation of level and the main features of rape seed quality. Sulfur and nitrogen as experimental factors effected at various way on examined features. Greater N dose and moderate S supply had the best effect on photosynthetic intensity and in a consequence final seed yield. Seeds originated from these objects were characterized with higher concentration and better quality of protein. However, higher seed yield production on objects with better N supply did not hold together with fat concentration and carbohydrates content in the leaves.

P8

Distribution of thiol compounds within fruits and vegetables and factors influencing their concentration

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Glutathione is multifunctional metabolite with numerous roles in cellular defence system and in sulphur metabolism. These functions depend or impact on the concentration and/or redox state of tissue glutathione pool [1]. Glutathione as other bioactives might be considered not only as component of plant defense mechanisms due to e.g. oxidative stress, but also in relation to its importance for human health. In our laboratory a comprehensive studies were made to test differences between genus, species and cultivars of horticultural crops, with respect to glutathione content, its redox state (GSH to GSSG ratio), the presence in the tested tissue of glutathione precursors such as L-cysteine and γ-glutamylcysteine and glutathione reductase activity, which reduce oxidized form of glutathione to reduced one. External factors that affected these components were: soil type and its fertility in relation to vegetables (lettuce, kale, cauliflower, broccoli, kohlrabi), storage type, time and distribution through fruit in *Malus* genetic resources. All studies were conducted through two/three growing seasons to asses impact of weather or other not-controlling environmental condition on aforementioned traits. Presented data are a certain compilation of published and non-published results.

A higher content of thiol compounds expressed sprouts or ripe vegetables, especially brassicas such as cauliflower (green cultivars), broccoli or kale as compared to the examined fruits (apple, blueberry). According to statistical evaluation apple glutathione content was highly tissue-type dependent, whereas concentration of L-cysteine and glutathione reductase activity were strongly influenced by condition of growing season. The content of γ-glutamylcysteine after harvest, especially in the examined fruits was very low and its level increased during storage. Great differences existed between examined cultivars of the tested fruits and vegetables. However in many cases the impact of growing season exceeded the genotype effect. Finally, the type of soil, time and storage condition dependent changes seemed to be of less important factors that affected thiol contents. Glutathione maintained its GSH/GSSG ratio with respect to both, fruits and vegetables tissue at a higher level, as compared to e.g. ascorbate.

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P9

Sulfur deprivation limits Fe-deficiency responses in tomato plants

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Ethylene has been suggested to play a role in the regulation of the response to Fe-deficiency in Strategy I plants, while nicotianamine (NA) acts as a chelator for internal Fe transport. Methionine requirement in ethylene and NA biosynthetic pathways suggests the involvement of plant sulfur nutritional status in the metabolic modifications necessary to cope with Fe shortage.
Seven-day-old hydroponically grown tomato (*Solanum lycopersicum* L. cv. Gimar) plants were transferred for a further week to a S-free nutrient solution (NS). Thereafter, half of the plants from the two S growth conditions (+S and –S), were transferred to a Fe-free NS for four days.
In +S plants, Fe deficiency caused an increase of the Fe(III)-chelate reductase activity, ^{59}Fe uptake rate and ethylene production at the root level. This response was further associated with increased expression of *LeFRO1* (Fe(III)-chelate reductase) and *LeIRT1* (Fe^{2+} transporter) genes. On the other hand when -S plants were transferred to a Fe-free solution, no induction of Fe(III)-chelate reductase activity and ethylene production was observed. The same hold true for *LeFRO1* gene expression, while increase of $^{59}Fe^{2+}$ uptake rate and *LeIRT1* gene over-expression were partially limited. Sulfur deficiency alone also caused a decrease in Fe content of tomato leaves and an increase of root ethylene production; however these events were not associated with either increased Fe(III)-chelate reductase activity, higher rates of ^{59}Fe uptake, or over-expression of both *LeFRO1* and *LeIRT1* genes.
Results show that S-deficiency could limit the capability of tomato plants to cope with Fe-shortage by preventing the induction of the Fe(III)-chelate reductase and limiting the activity and expression of the Fe^{2+} transporter. Furthermore, results support the idea that ethylene alone can not trigger specific Fe-deficiency physiological responses in a Strategy I plant, such as tomato.

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P10

Impact of copper on growth, sulfate uptake and assimilation in *Brassica pekinensis*

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Copper is an essential micro-nutrient for plant growth, which is potentially toxic at supra-optimal levels. Cu^{2+} may react with sulfur metabolites and in addition it may induce the formation of phytochelatins. Seedlings of Chinese cabbage (*Brassica pekinensis*) were grown at levels ranging from 1 to 10 μM Cu^{2+} for one week, which resulted in a strongly increased level of water-soluble non-protein thiols in the root and slight increase of that in the shoot with concentration. The plant biomass production and nitrate uptake by the root were decreased at > 2 μM Cu^{2+}, whereas the sulfate uptake (and sulfate uptake capacity) was slightly enhanced at 2 to 5 μM Cu^{2+}. The latter was accompanied with an increase in total sulfur content of the shoot, which could pre-dominantly be ascribed to an accumulation of sulfate. The significance of the observed effects of Cu^{2+} on sulfur metabolism of Chinese cabbage will be evaluated.

P11

Affecting of plants by silver ions revealed by electrochemical and spectral techniques

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The ions of heavy metals and their compounds are considered as one of the most toxic substances polluting all parts of environment. Photographical industry, following electrochemistry and medicine are the main sources of one of the most toxic heavy metal ions, silver(I) ions. The aim of this work is to investigate sunflower plants response on stress induced by various doses of silver(I) ions (0, 0.1, 0.5, and 1 mM). For this purpose we employed multi-instrumental apparatus to detect and investigate total protein content, urease activity, spatial distribution of the heavy metal ions, and physiological and anatomical changes in the treated plants (sunflower, maize, early somatic embryos).

The plants were treated with silver(I) ions for 96 h and sampled per 24 hours of the treatment. We found that the treated plants embodied growth depression, coloured changes and lack root hairs. Using of autofluorescence of anatomical structures, such as lignified cell walls, it was possible to determine the changes of important shoot and root structures, mainly vascular bungles and development of secondary thickening. The differences in vascular bundles organisation, parenchymatic pith development in the root centre and the reduction of phloem part of vascular bundles were well observable. At early somatic embryos the growth depression was also well apparent. Further we employed laser induced breakdown spectroscopy for determination of spatial distribution of silver(I) ions in tissues of the treated plants [1]. The Ag is accumulated mainly in near-root part of the sample. Moreover basic biochemical indicators of environmental stress were investigated. The total content of proteins expressively decreased with increasing silver(I) ions dose and the time of the treatment. This phenomenon can be related with the cascade of processes connecting with photosynthesis. Finally we studied the effect of silver(I) ions on activity of urease in *in vitro* conditions.

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P12

Interactions between chromate and sulfate affect growth, photosynthesis and ultrastructure in *Brassica juncea*

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Previuos studies provided evidences that chromate and sulfate compete for the transport into the cells [1,2]. To elucidate the physiological effects of the sulfate and chromate interactions, *Brassica juncea* plants were grown for 96 h under the following conditions: no sulfate and no chromate (-S), no sulfate plus 0.2 mM chromate (-S + Cr), 0.2 mM sulfate (+S), 0.2 mM sulfate plus 0.2 mM chromate (+S +Cr). The growth of plants was affected by chromate in terms of biomass fresh weight and root length. The content of chlorophylls was similar among plants of the conditions –S, -S +Cr, +S +Cr, and was significantly lower than that recorded in +S plants. The levels of photosynthetic activity, transpiration and stomatal conductance were drastically reduced following plant exposure to chromate. In particular, stomatal conductance was more affected by metal treatment with a reduction of about 80%. Chromate also caused alterations of the cell structure of the spongy and palisade parenchyma, modifications of the stroma thylakoids and formation of vesiculations in the stroma. The accumulation of chromium in leaves and roots was time-dependent and was higher at 72 h and 96 h in –S + Cr plants compared to plants of the condition +S + Cr. The level of sulfur decreased in +S plant after 72 h of Cr treatment and in leaves was comparable to that measured in plants of the other three experimental conditions. Plants of the condition –S + Cr tended to maintain higher sulfate pools compared to the –S plants, in both roots and leaves, while in +S plants the content of sulfate in roots decreased during Cr treatment.
From these results we can conclude that the interactions between sulfate and chromate must be taken into consideration when plants are going to be employed for the remediation of chromate contaminated sites, as the accumulation of Cr in plant tissues might be altered by the sulfate concentration in the substrate where plants grow.

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P13

Overexpression of phytochelatin synthase affects sulfur metabolism in tobacco plants both under cadmium and arsenate exposure

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Phytochelatins (Cys-rich heavy metal complexing peptides) play an important role in constitutive cadmium and arsenate tolerance. They are synthesized by phytochelatin synthase (PCS, EC 2.3.2.15) from glutathione. A previous study by Li *et al.* [1] on *Arabidopsis* demonstrated contrasting responses to cadmium and arsenate due to PCS overexpression: cadmium hypersensitivity along with an increased arsenic tolerance; however, the reason for this difference remains unknown. Our study on a model plant species tobacco transformed with two different phytochelatin synthase genes: *AtPCS1* and *CePCS* addresses the mechanisms underlying the variation in response to cadmium and arsenate reported for *PCS* overexpressing plants. We demonstrated that *CePCS* transformants were more tolerant to Cd^{2+} than WT, whereas *AtPCS1* expressing plants were Cd-hypersensitive. However, no substantial difference in Cd accumulation between studied lines was detected. *PCS* overexpressing plants differed in the non-protein thiol profile both when exposed to cadmium (3 days, 5 and 25 μM) and arsenate (V) (3 days, 200 μM). *AtPCS1* transformants displayed a dramatic accumulation of γ-glutamylcysteine and concomitant strong depletion of glutathione both in roots and leaves under cadmium exposure, but only in arsenate treated roots. By contrast, in *CePCS* transformants, a smaller reduction of the level of glutathione was noticed along with less pronounced changes in γ-glutamylcysteine concentration. Surprisingly, the phytochelatin levels did not increase significantly due to *AtPCS1* overexpression despite the 5-fold higher PCS activity compared to WT plants. On the other hand, moderate increase in *PCS* activity in CePCS1 transformants (~40%) resulted in an increase of PC level but only in cadmium exposed roots. Our results clearly demonstrate that overexpression of the single PCS gene can strongly interfere in related sulfur metabolic pathways, leading to results opposite to those expected, and that those effects depend on the *PCS* gene used for the transformation.

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P14

Analysis of phytochelatin and phytochelatin synthase using liquid chromatography with electrochemical detection

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Heavy metals are considered to be dangerous pollutants due to their toxic effects to all organisms. Most of the methods used for remediation of polluted environment suffer from high costs. One of the alternative way represents phytoremediation [1]. This process describes the treatment of environmental problems (bioremediation) through the use of plants.

When heavy metals enter into a plant cell, they initiate the biosynthesis of proteins rich in sulfur called phytochelatins, which are used for heavy metal detoxification. Phytochelatins (PC) are cysteine-rich small peptides consist of 4-23 amino acids, which have a basic formula (γ-Glu-Cys)n-Gly (n = 2 to 11). The synthesis of phytochelatins proceeds from glutahione by transferring a γ-Glu-Cys moiety from a donor to an acceptor molecule. The reaction is catalyzed by γ-Glu-Cys dipeptidyl transpeptidase (EC 2.3.2.15), which has been called as phytochelatin synthase (PCS). *In vitro* the activity of the partially purified enzyme was active only in the presence of metal ions. The best activator PCS tested was cadmium followed by silver, bismuth, lead, zinc, copper, mercury, and gold cations.

In the present work we investigated the influence of cadmium(II) ions on phytochelatin synthase activity and on the total phytochelatins content in the treated early somatic embryos of blue spruce (*Picea pungens* Engelm.). For these purposes the high performed liquid chromatography with electrochemical detector (HPLC-ED) was used. Using this method we focused on the detection of phytochelatin 2 (PC2), 3 (PC3), 4 (PC4) and 5 (PC5). It clearly follows from the results obtained that the content of PC2, PC3 and PC5 enhanced till the end of treatment. However the content of PC4 in th treated embryos decreased. Moreover we used the total contents of phytochelatins to measure activity of phytochelatin synthase in the early somatic embryos of spruce treated with the heavy metal ions. Particularly the method is based on the determination of the total content of phytochelatins in reaction mixtures with real plant sample, where defined concentration of heavy metal is introduced. Based on the content of phytochelatins synthesized we were able to determine the phytochelatin synthase activity, or more precisely, the rate of phytochelatins synthesis. We determined that the total phytochelatin synthase activity enhanced not only with the increasing dose of cadmium(II) ions, but also with the time of the treatment.

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P15

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, thyroid dysfunction, and reduced immune functions [1]. 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 [2]. The high affinity Group 1 type contains the main transporters involved in the uptake of sulphate by roots and therefore most likely selenate. To investigate the role of sulphate transporters in selenate uptake, cDNAs for sulphate transporters were cloned from both selenium hyper-accumulating species (*Astragalus racemosus*, *Astragalus bisulcatus, Astragalus crotalariae*) and closely related non-accumulating species (*Astragalus sinicus*, *Astragalus glycyphyllos, Astragalus drummondii*) by RACE. Bioinformatic analysis of the sequences indicated homology to the Group 1 type of sulphate transporters genes. Multiple isoforms of this transporter gene were identified for each species. Sequence variation has the potential to modify the ratio of selectivity of sulphate and selenate transport. Full length *Astragalus* transporters were further cloned in a yeast vector and transformed into a yeast mutant deficient in sulphate transport ability to facilitate functional analysis.

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P16

Cysteine biosynthesis in *Arabidopsis*: comprehensive study on the functions of *Serat* and *Bsas* gene families

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The final steps in Cysteine (Cys) biosynthesis are catalyzed by two enzymes, serine acetyltransferase (Serat) and *O*-acetylserine (thiol) lyase (OASTL) which is classified within β-substituted alanine synthase (Bsas) family. Serat catalyzes the formation of *O*-acetylserine (OAS) from acetyl-CoA and serine. OASTL catalyzes the formation of Cys by the incorporation of the reduced sulfide into OAS. By contrast to sulfate reduction which takes place mainly in the plastids, both Serat and OASTL enzymes were found in plastids but also cytosol and mitochondria of plant cells. In *Arabidopsis*, *Serat* and *Bsas* gene families comprise five members and nine members, respectively. Until now, the biochemical characterizations in vitro and subcellular localization studies of the members in both families were partially conducted, but the difference of contribution for OAS and Cys formation of each member in vivo and the significance of subcellular compartmentation of Cys synthesis have remained unaddressed. To address these questions, we performed the comprehensive analysis of knockout mutants of all members in *Bsas* and *Serat* genes families. The analysis of the *bsas* single-mutants [1] revealed that cytosolic Bsas1;1 has the most dominant contribution for Cys formation in leaf and root, and mitochondrial Bsas2;2 play a significant role in root. Plastidic Bsas2;1 contributes the cellular OASTL activity, but no alternation in thiols contents in leaf and root of *bsas2;1* mutant was observed. On *Serat* gene family, we analyzed the multiple-knockout mutants besides single-knockout mutants. The quintuple mutant showed embryo-lethal phenotype, but all five quadruple mutants remaining a single each gene could survive, indicating that all five isoforms are functional in vivo, and no other pathway operates besides *Serat* gene family for Cys synthesis. The analysis of the *serat* mutants demonstrated that mitochondrial Serat2;2 plays the predominant role, and plastidic Serat2;1 participates to a lesser extent for OAS formation in vivo. The cytosolic isoforms, Serat1;1, Seat3;1, and Serat3;2, may play a major role during seed development. The results from analyses of *bsas* and *serat* mutants suggested that Cys synthesis in plastids was not carried out predominantly in contrast to what has been believed in *Arabidopsis*, and Cys formation might be mainly performed in cytosol using free sulfide released from plastids and sufficient OAS released from the mitochondria.

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P17

How does sulphur availability modify N acquisition by White Clover (*Trifolium repens* L.)?

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During the last three decades, atmospheric sulphur deposition has decreased dramatically [1, 2]. This induced a sulphur impoverishment of soils in Northern Europe [2] and sulphur (S) deficiencies begin to appear in grassland herbages. This change could modify specific composition and productivity of grasslands. S is essential for plants, particularly for leguminous species because of its suspected effect upon nitrogen fixation. The hypothesis of this study was that good sulphur availability improves White Clover performances mainly by increasing N_2-fixation. The aim of our study was to identify the mechanisms which allow S to increase N_2 fixation in White Clover. Plants of *Trifolium repens* cv huia were grown during 140 days, in a hydroponic system and in condition of N_2-fixation inhibition (high availability in Nitrate). Three treatments have been chosen "No S", "Low S" (0.095 mM SO_4^{2-}) and "Optimum S" (0.380 mM SO_4^{2-}). NO_3^- absorption and N_2-fixation were measured with the isotopic dilution method by the use of nutrient solution enriched with ^{15}N (0.5 %).

S availability modified significantly White Clover performances. An increase of S availability induced an increasing dry mass production. As expected S availability allowed a best N acquisition by increasing atmospheric N_2-fixation as the proportion of N coming from N_2-fixation increased with an increasing S concentration. While N absorption increased quite proportionally with root biomass. Morphological parameters of nodule analysis revealed that increasing [SO_4^{2-}] in nutrient solution increased nodulation: biggest nodule dry mass, number and volume for root unit were observed.

Our study shows that S availability induces better N_2-fixation by increasing nodulation. Effect of S availability on nodule proteins (nitrogenase and leghemoglobin) remains to be studied.

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Key words: Sulphur availability, N_2-fixation, NO_3^- absorption, Nodules, White Clover.

P18

Possible connection of sulfur and C2-subunit metabolism by N-terminal acetylation of proteins

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The enzyme *O*-acetylserine(thiol)lyase (OAS-TL) can limit the synthesis of cysteine in the cytosol of particular cell types and under certain stress conditions in general [1]. Proteins that catalyze rate-limiting reactions are often targets for regulation by co- and post-translational mechanisms.

A common protein modification in the cytosol of eukaryotes is N-terminal acetylation (NTA), which occurs co-translationally and can modify protein function, protein-protein interaction and thermal stability [2]. N-terminal acetylation is an enzyme-catalyzed reaction in which peptide alpha-N-acetyltransferases (PNA) transfer the acetyl group from acetyl-coenzyme A to the α-amino group of protein.

The best analysed organism with respect to NTA is *Saccharomyces cerevisiae* that contains three different cytosolic isoforms of PNA: PNA-A, PNA-B and PNA-C. The only characterised PNA of higher plants (At2g38130, AtMAK3) is the ortholog of yeast Mak3p, which encodes the catalytic subunit of PNA-C [2].

The aim of this study is to identify ortholog of yeast PNAs in the genome of *Arabidopsis thaliana*. A blast search using amino acid sequences of the catalytic subunits of PNA-A (ARD1p) and PNA-B (NAT3p) of yeast reveals Arabidopsis proteins that have sequences similarities between 30-50% towards the yeast PNA. A number of genetic and biochemical approaches will be combined to confirm the biochemical identity of the putative Arabidopsis PNA proteins.

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P19

A $^{34}SO_4^{2-}$ pulse-chase labeling method to study the S recycling in oilseed rape submitted to SO_4^{2-} deficiency during the rosette stage.

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The decrease of sulphate availability in soil, which is largely due to the decline of industrial rejections of SO_2, alters both grain yield and oil quality of oilseed rape (*Brassica napus* L.). Consequently, S fertilization is now recommended in many countries. In order to optimize the S fertilization (adjustment of the period and level of S fertilization to S demand of crop), it will be necessary i) to characterize the stages of crop cycle that are the most affected by S deficiency and ii) to understand the mechanisms which contributed to an efficient recycling of S compounds from source to sink tissues. In this context, our aim was to determine firstly if the rosette stage is a vegetative phase of development particularly affected by a transient S deficiency. To assess the S deficiency effects, a method of $^{34}SO_4^{2-}$ pulse-chase labeling was used.

Fifteen days after sowing, plantlets were transferred in hydroponic conditions and labeled with 0.3 mM of $^{34}SO_4^{2-}$. After 50 days, the labeling was stopped and plants were submitted to two SO_4^{2-} treatments during 35 days: High S (HS) *versus* Low S (LS, 20 fold lower than HS). The incidence of SO_4^{2-} deficiency on S and ^{34}S remobilization from source leaves was studied using isotope ratio mass spectrometry. These data were compared to the expression of the *BnSultr4;1* (a gene encoding a vacuolar SO_4^{2-} transporter implicated in SO_4^{2-} efflux). The senescence progression was studied using an accurate molecular indicator of leaf senescence status (*SAG12*/*Cab*) [1].

At rosette stage, the growth of young leaves in LS plants is significantly reduced only after 35 days compared to HS plants. After 35 days, the total S and SO_4^{2-} in maturating leaf of LS plants is 4 fold lower than HS plants while the soluble protein amount remains similar to HS plants. The ^{34}S amount in maturating leaf of LS plants rapidly declines and is 2 fold lower than HS plants after 35 days. This large decline of ^{34}S amount was associated with an induction of the expression of *BnSultr4;1* suggesting that vacuolar SO_4^{2-} is specifically remobilized to sustain the S demand for growth. It is concluded that when transient mineral S deficiency occurs at rosette stage, oilseed rape is able to maintain leaf growth by an optimization of the recycling of endogenous S compounds (especially SO_4^{2-}) without any acceleration of leaf senescence process.

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P20

Sulphur Metabolism of Marine Phytoplankton: Biochemical Pathway to Climate Cooling

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The production of dimethylsulphide (DMS) by marine phytoplankton plays a key role in the global sulphur cycle. The sea-to-air flux of this volatile compound transfers sulphur from the oceans, which are a major sulphur reservoir, to the relatively sulphur-limited land. Furthermore, DMS oxidises in the atmosphere to form aerosol particles that have a cooling affect on the climate, directly through the refection of solar radiation and indirectly through the formation of cloud condensation nuclei.

DMS is the breakdown product of dimethylsulphoniopropionate (DMSP), found in various species of phytoplankton. Despite its importance little is known about the regulation of DMSP production. There has been little research into the basic sulphur metabolism in marine phytoplankton as sulphur is assumed not to be limiting in the ocean. Most of our knowledge of the mechanism and control of sulphur assimilation is derived from experiments with higher plants.

In recent years there have been a number of breakthroughs in algal genomics, with species such as the freshwater green alga *Chlamydomonas reinhardtii* and the diatom *Thalassiosira pseudonana* being fully sequenced. This provides a huge bioinformatic resource, which will enable new approaches to algal biology. We have already discovered novel variants of enzymes in the sulphate assimilation pathway, which could provide insight into the evolution of higher plants.

We want to use biochemical and molecular approaches to investigate sulphate assimilation and DMSP production in marine phytoplankton with the aim of determining which biochemical steps control the rate of DMSP production. This will increase our understanding of the processes that control DMSP production by phytoplankton in the marine environment and could contribute to future climate and biogeochemical models that incorporate production of the climate-cooling gas DMS. Here we outline the project and present our first findings on DMSP production and APS reductase activity in *T. pseudonana*.

P21

The homocysteine regulon in *Aspergillus nidulans*

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Homocysteine is an intermediary amino acid involved in methionine, cysteine, and S-adenosylmethionine metabolism. An excess of homocysteine is harmful for animal and yeast cells. Therefore, homocysteine level is usually kept low by remethylation to methionine catalyzed by methionine synthase (MS) in a reaction that requires folate (MTHF). Homocysteine can also be catabolized to cysteine in the transsulfuration pathway involving cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL). Impaired activity of either of these pathways results in the accumulation of homocysteine. *Aspergillus nidulans* mutants impaired in CBS are inhibited by an exogenous methionine or homocysteine which suggests that homocysteine may be toxic also to filamentous fungi.

We found that several *A. nidulans* genes are regulated by an exogenous homocysteine. The homocysteine-induced genes encode enzymes that metabolize homocysteine including CBS, CGL and MS [1]. Some of these genes encode enzymes of the folate cycle – *e.g.* MTHFR (methylenetetrahydrofolate reductase which synthesizes MTHF) [2] , FPGS (folylpolyglutamate synthase) and DHFS (dihydrofolate synthase) as well as methionine catabolic enzymes – S-adenosylmethionine synthase (MAT) and S-adenosylhomocysteine hydrolase (SAH).

We conclude that a new regulatory system which we call the "homocysteine regulon" was identified in *A. nidulans*. This regulatory system controls genes that participate in the conversion of homocysteine to less harmful sulfur amino acids.

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P22

Investigation of inhibition of glutathione biosynthesis at early somatic embryos of Spruce

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Glutathione (GSH) is a tripeptide consisting of cysteine, L-glutamate and glycine. Glutathione can be considered as ubiquitous molecule because of its presence in all living organisms even at units of mM [1]. Detoxifying of some xenobiotics and heavy metals and marinating of the redox pool belong to the most crucial functions of GSH. Moreover GSH can be a substrate for more complex peptides called phytochelatins, which are synthesized to protect a plant cell against heavy metals. The main aim of this work was to investigate the effect of glutathione biosynthesis inhibitor buthione sulfoximine (BSO) on total content of mRNA at early somatic embryos of Spruce.

The cultures of early somatic embryos of Norway Spruce (ESEs), clone designated as 2/32, were maintained on a liquid half-strength LP medium in Erlenmeyer flasks placed in shaker (110 rpm) in dark at 25 °C. Various concentrations of BSO (0, 50, 100, 250 and 500 μM) were added to the cultivation media containing ESEs. The ESEs was exposed for eleven days and sampled at 0, 2, 4, 7, 9 and 11th day. Moreover ESEs were treated with BSO and cadmium(II) ions at concentrations of both 0, 50, 250 and 500 μM.

Primarily we investigated the influence of BSO on the total content of mRNA –transcriptome at ESEs. We found that the level of total mRNA enhanced for more than 10 % at BSO treated ESEs compared to control embryos. At the end of the treatment the highest BSO dose (500 μM) stimulated synthesis of mRNA four times compared to control embryos. Furthermore we aimed our attention on ESEs treated with BSO + cadmium(II) ions. The highest enhancing of transcriptome level was determined at ESEs treated with 500 μM of cadmium(II) ions. Four days after application of the highest dose of BSO + cadmium(II) ions the level of transcriptome increased three times compared to control ones. This results encouraged us to detect glutathione at BSO and BSO + cadmium(II) ions treated embryos. It clearly follows from the results obtained that BSO addition resulted in moderate enhancement of GSH level at all experimental groups till fourth day of the treatment. This phenomenon can be related with GSH release from conjugates such as GSH-saccharide and others. After seven days of the treatment the GSH level at the BSO treated ESEs was lower compared to the control ones. BSO + cadmium(II) ions effect led to increase of GSH level till 9th day of the treatment and then the GSH level decreased compared to the control ESEs.

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P23

LL-DAP-aminotransferase and threonine synthase temporal expression in developing quality protein maize seeds

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Cereals typically provide ~ 50% of the dietary protein for humans and can comprise up to 70% of the protein intake [1]. However, the most abundant storage proteins they contain, the prolamin storage proteins known as zeins in maize, are poor in lysine, an essential amino acid [2]. To better understand some of the key enzymes controlling lysine and threonine metabolism in the maize endosperm during development, we performed relative quantification gene expression of *LL-DAP aminotransferase* (*LL-DAP-AT*) and *Threonine synthase* (*TS*). Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was selected as reference gene. The plant material used were a wild type maize line (L161) and two Quality Protein Maize (QPM) lines (L161o and L161q). The QPM lines are the 6^{th} generation of modified backcross with 96.875% wild type recovery and have different seed vitreous aspects; L161o grains are whole opaque, while L161q seeds are vitreous-top and opaque-bottom. In plants, aspartate serves as a precursor for the synthesis of lysine, methionine, threonine and isoleucine in the aspartate pathway [3]. The LL-DAP-AT is the most recent enzyme identified in the lysine biosynthesis branch [4]. TS is involved in the branching point between the methionine and threonine biosynthesis. In developing seeds, *LL-DAP-AT* was induced in both QPM lines at 14 days after pollination (DAP), and an enhanced expression was observed at 20 DAP in L161o. At 24 DAP, the line L161q exhibited up-regulation for both analyzed genes and *LL-DAP-AT* was three times more expressed than *TS*. These very first results for high-lysine seeds are an important part of a broader picture to understand the mechanisms responsible for increased protein quality in the QPM varieties that would enable more rapid and significant improvement of maize nutritional quality.

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P24

Does mineral S availability alter S and ^{34}S dynamics during vegetative growth of rapeseed?

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In higher plants, sulphur (S) is an essential element for crop yield and quality [1]. However, S availability has been decreasing in many areas of Europe since last decade [2], which severely reduced the yield by more than 40% [3]. Rapeseed (*Brassica napus* L.) is a plant of worldwide importance and it requires high inputs of S fertilizers. This plant is particularly sensitive to S deficiency because it has a high demand for S [4] in order to produce seeds with a high yield of protein with relatively large quantities of S-containing amino acids [5]. Even if the importance of S was well documented since many years [6], the physiological effects of S deficiency remain largely unclear. As a consequence, we studied the effects of mineral S deficiency on S fluxes during vegetative growth of rapeseed at both whole plant and leaf rank level (*i.e.* leaf tissues representing more than 80% of total biomass; data not shown).

Rapeseed plants were sown and grown in a greenhouse during six weeks in hydroponics with optimal N and with 300 µM $^{34}SO_4^{2-}$. At this date, two treatments were applied during 35 days with 300 µM $^{34}SO_4^{2-}$ for control plants (+S) or with 15 µM $^{34}SO_4^{2-}$ for S deficient plants (-S). Natural light was supplemented with phytor lamps (150 µmoles.m-2.s-1 of photosynthetically-active radiation) for 16 h with a thermoperiod of 24°C (day) and 18 °C (night).

Our results highlight that S deficient plants showed no significant differences either on whole plant and leaf rank biomasses, when compared to control plants. However, either for whole plant and leaf ranks total S and ^{34}S (*i.e.* deriving from S uptake) amounts are greatly reduced after 35 days. For example, plant total S amount was decreased from 159 mg. plant^{-1} for control plants to 57 mg. plant^{-1} for S deficient plants.

Even if S deficient plants had 20 times less mineral S than control plants, and therefore presents contrasted S managements (total S and ^{34}S), their development remained surprisingly unchanged. This could be due to the plant high initial S level (*i.e.* S reserves). As a conclusion, during its vegetative growth, our results highlight that rapeseed presents a great physiological adaptation through the fine management of S fluxes within the plant. As suggested by Gleeson [7], this adaptation is mediated by optimization of S cycling within the plant. However, and because S deficiency can reduce the yield by more than 40% [3], this great adaptation should be solely effective on a short time scale (i.e. vegetative growth).

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P25

Identification and regulation of *Chlamydomonas* sulfate transporters

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Sulfur is an essential element present in proteins, lipids, carbohydrates, and several metabolites. For many organisms, sulfate is the preferred source of sulfur, and it is transported into the cytosol of cells by specific anion transport proteins. In photosynthetic organisms, the sulfate that enters the cells can be reduced to sulfide in the chloroplasts, with subsequent incorporation into the amino acids cysteine and methionine and into key metabolites such as glutathione and phytochelatins. Analysis of the *Chlamydomonas reinhardtii* genome has led to the identification of five genes that encode proteins with high sequence similarity to known sulfate transporters. Two of these transporters, SULTR1 and SULTR2, exhibit strong sequence similarity with H^+/SO_4^{-2} co-transporters which are typical of vascular plants, while the remaining three, SLT1, SLT2, and SLT3, exhibit strong sequence similarity to the Na^+/SO_4^{-2} transporters that have been identifed in animals and microbes, but not in plants. The expression patterns of these putative sulfate transporters were examined under sulfur-replete and sulfur-deplete conditions using quantitative real time PCR. Transcripts from *SULTR2, SLT1,* and *SLT2* rapidly increased when the cells were deprived of sulfur, whereas those of *SULTR1* and *SLT3* decreased. Increases in *SULTR2* and *SLT* transcript abundance were correlated with large increases in the accumlation of the encoded transport proteins. Furthermore, changes in the abundance of transcripts for the various sulfate transport proteins was demonstrated to be under the control of SAC1 and SNRK2.2, key regulators of the responses of *C. reinhardtii* to sulfur deprivation. Both the sulfate transporter proteins and the RNA encoding these proteins are rapidly lost from sulfur-deprived cells following administration of sulfate to those cells. Our results suggest that SULTR2, SLT1 and SLT2 contibute to the high affinity transport that develops when *C. reinhardtii* is starved for sulfur and that the abundance of these transporters is controlled by a phosphorelay involving SAC1 and SNRK2.2.

Thus far, we have not been able to complement a sulfate transporter-deficient strain of *Saccharomyces cerevisiae* or a sulfate transporter mutant in *Arabidopsis thaliana (sel1-9* strain) with the *SULTR2, SLT1* or *SLT2* gene; this may be a consequence of the lack of proper activation/modification/trafficking of the encoded transporters. In an attempt to demonstrate the function of the putative transport proteins, we are screening for *C. reinhardtii* mutants harboring an insertion in the genes encoding these proteins.

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Post-transctiprional control of high-affinity sulfate transporters for uptake of sulfate in Arabidopsis roots

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Induction of sulfate uptake is the primary response to sulfur limitation. In *Arabidopsis thaliana*, two high-affinity sulfate transporters SULTR1;1 and SULTR1;2 are expressed at the epidermis and cortex of roots, and their mRNA levels are upregulated during sulfur limitation (-S). The *sultr1;1 sultr1;2* double knockout mutant (*DKO*) completely lacked sulfate uptake capacity and showed severe growth defects under the low-sulfate conditions. In contrast to *DKO*, both *sultr1;1* mutant and *sultr1;2* mutant retained substantial capacities to take up sulfate from the same low-sulfate conditions, suggesting that SULTR1;1 and SULTR1;2 can act independently for the acquisition of external sulfate.
To study post-transcriptional regulation of SULTR1;1 and SULTR1;2 in response to -S, we generated transgenic plants overexpressing SULTR1;1mycHis and SULTR1;2mycHis epitope-tagged sulfate transporters under the control of cauliflower mosaic virus 35S promoter using *DKO* as a parental line [1]. Expression of SULTR1;1mycHis and SULTR1;2mycHis, respectively, rescued the growth of *DKO* under low-sulfate conditions. Although 35S promoter is suggested to be constitutively active irrespective of plant organs, both *SULTR1;1mycHis* and *SULTR1;2mycHis* mRNAs accumulated predominantly in roots. SULTR1;1mycHis and SULTR1;2mycHis proteins were expressed exclusively in roots and started to accumulate no later than 8 hours after withdrawal of sulfate from the medium, whereas the levels of their corresponding transcripts showed no significant change under the same conditions. It is suggested that the accumulation of SULTR1;1mycHis and SULTR1;2mycHis proteins during -S is attributable to increased translation and/or changes in protein stability. In parallel with the increase of SULTR1;1mycHis and SULTR1;2mycHis protein levels, sulfate uptake capacity of both transgenic lines considerably increased by -S. The present study suggest the existence of post-transcriptional mechanisms for the control of SULTR1;1 and SULTR1;2, in addition to the previously reported transcriptional regulation.

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P27

The influence of sulphur depletion on the expression of sulphur metabolism related genes and on the phytohormone profile of poplars (*Populus tremula x P. alba*)

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Sulphur is taken up by plants mainly from the soil in the form of sulphate. This is largely stored in the vacuoles of root cells or loaded into the xylem and allocated to the leaves with the transpiration stream. There it is reduced and assimilated into cysteine. In response to sulphur availability, a demand-driven regulation of sulphate uptake by interorgan translocation of sulphate and reduced sulphur compounds such as glutathione via phloem transport has been postulated [1, 2]. In *Arabidopsis*, also cytokinins are involved in balancing sulphur homoeostasis. The high-affinity sulphate transporters *AtSULTR1;2* and *AtSULTR1;1* from *Arabidopsis* are down-regulated by cytokinins accompanied by a decrease in sulphate uptake [3]. We analysed the gene expression of different sulphate transporters and enzymes involved in sulphur assimilation in roots and leaves of poplars grown on sand watered with a nutrient solution lacking sulphur. Among the analysed genes, the sulphate transporter *PtaSULTR1;2,* which is expressed only in roots, reacts first on the decreasing sulphur availability. At the time-point of this first reaction, we analysed the phytohormone profile in leaves, roots and transport tissues compared with normal sulphur nutrition.

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P28

Influence of short term sulfur starvation on polyprenols level and photosynthesis in tobacco

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Sulfur starvation affects variety of plants processes including photosynthesis and on the other hand, reductive assimilation pathway of sulfate is influenced by availability of photosynthetic electrons and carbohydrates, which fluctuates diurnally [1]. Evident proof for such interactions is chlorosis occurring on the leaves of sulfur starved plants that are producing insufficient amount of chlorophyll and lipids, what in turn results in reduction of photosynthetic activity [2].
Results obtained for 2-days starved tobacco plants with SSH method revealed that several genes related to photosynthesis were differentially regulated by sulfur deficit [3]. Additional screening allowed for identification of other genes encoding proteins involved in this process. This result showing influence of sulfur limitation on expression of genes encoding proteins connected with photosynthesis is consistent with results obtained for *Arabidopsis* [4]. Further experiments with tobacco have shown influence of short term sulfur deprivation on amount of chlorophylls, carotenoids, and pool of other isoprenoids derivatives such as plastoquinone, playing important roles as an electron carries in the light-dependent reaction of photosynthesis, and in regulation of gene expression, and solanesol, a main polyprenol in tobacco with less known function. In spite of such apparent changes, results from photosynthethetic activity measurements indicated that two days sulfur starvation did not made any significant damages in photosynthetic apparatus. We suppose that plants at such an early step of sulfur starvation are able to overcome the stress through modifications of the chloroplast processes.

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P29

Effects of sulfate-deprivation on β-galactosidase, β-glucosidase, pectin-methylesterase, and pectin-acetylesterase gene expression in maize root types

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The cell wall comprises a complex system of polysaccharides with incorporated stuctural proteins, enzymes and phenolic substances. The structure of the cell wall varies due to targeted changes that modify its components and the plasticity of cell wall, which is a prerequisite for the elongation of cell and the resulting plant growth. It is evident that a large number of genes are involved in the biosynthesis and modification of the cell wall [1]. In the present work the levels of expression of beta-galactosidase (EC 3.2.1.23), beta-glucosidase (EC 3.2.1.21), pectin-methylesterase (EC 3.1.1.11) and pectin-acetylesterase (EC 3.1.1) genes that display hydrolytic activity against cell wall components were studied in the root system of maize plants cultivated in a hydroponic system with S-sufficient and S-deficient nutrient solutions. The expression of the selected genes was monitored by means of real time PCR in the primary root, the seminal roots, and the 1st nodal roots of maize seedlings, focusing on two sectors [2]: the apical one (A), and the ELR where the growth of lateral roots has been activated. To this end, 10-days-old maize seedlings were subjected to S-deprivation for the next 12 days (period chosen because the 1st whorl of nodal roots emerge at this stage and are exposed to S-deprivation throughout development).

When the plants were grown in the complete nutrient solution, beta-galactosidase was expessed at higher levels in sector A of the primary root and in the nodal roots of first whorl compared with the ELR sector. The same profile was observed for beta-glucosidase in the embryonic roots, whilst the expression of the pectin-methylesterase gene increased, particularly during the initial days of development, in both sectors of the primary root. The timing of gene expression under a complete nutrient solution showed that the maximum expression of pectin-methylesterase preceded the beta-galactosidase. Under sulfate deprivation, the expression levels of beta-galactosidase were lower compared with the controls. Conversely, beta-glucosidase expression was significantly higher compared with the controls. The expression of pectin-methylesterase was higher in ELR of primary roots, while pectin-acetylesterase was mainly expressed in the A sector. Under sulfate deprivation, the maximum expression of all cell wall degrading genes studied took place at day 6 of the treatment, while under complete nutrition conditions such a pattern was not observed.

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Effect of sulfate-deprivation on pectins of maize nodal roots

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10-days-old maize seedlings were subject to S-deprivation for 12 days, and the 1st nodal roots which belong to the post-embryonic system of maize roots [1] were examined for alterations in pectins using *in situ* approaches. Ruthenium red has been used to reveal the *in situ* distribution of low esterified pectins (LEP), and the monoclonal antibodies Jim5, Jim7 [2], LM5 [3] and LM9 [4] were used for the localization of no- or low-esterified homogalacturonans (LEH), of highly- esterified homogalacturonans (HEH), of rhamogalacturonans with neutral galactan side chains (NRG), and of rhamogalacturonans with feruloyl galactans in side chains (FRG) respectively. Electron microscopy was focused on the study of the appearance of cell walls, especially the junctions. Based on the presence or absence of lateral roots, four sectors were distinguished and examined: the basal (B) sector carrying no laterals, the (LR) sector carrying the growing laterals, the (ELR) sector carrying the emerging laterals, and the apex (A) sector carrying no laterals, which includes root elongation zone [5].
S-deprivation resulted in specific changes in each root tissue. In the rhizodermis LEH, HEH and FRG did not exist. LEP decreased in A and ELR, disappeared in LR and B at d6 and increased in B at d12, whilst an increase in NRG was located in B at d12. In the three layers of the hypodermis FRG did not exist, whilst LEH was found in ELR at d12 and in B at d6. LEP decreased in A and ELR, and increased in LR and B at d12. HEH increased 1cm from root tip and in B at d6. NRG were located in B at d6. In the cortex, LEH were found in the junctions of ELR, LR, and B at d6, and extended in the cell walls at d12. There were no changes in LEP and FRG, whilst NRG did not exist. HEH increased in the junctions of A, ELR and B at d12. In the endodermis LEH were found in ELR at d6, LEP decreased in B at d12, HEH increased in ELR at d6 and d12, whilst NRG and FRG did not exist. Thus, it is concluded that pectin esterification, as well as feruloylation of galactan side chains of root cell walls are subject of modification under S-deprivation. Furthermore this modification is tissue specific and connected with the presence (or absence) of lateral roots.

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Analysis of mutants with altered responses to sulfur deficiency

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To study the regulatory mechanisms of sulfur-deficiency (-S) responses in plants, we have isolated and analyzed mutants with different patterns of gene expressions in response to -S [1, 2]. As a parental line, we used transgenic *Arabidopsis thaliana* expressing GFP reporter gene under control of a -S-responsive chimeric promoter [3]. Here we report the isolation and characterization of seven mutants with altered responses to -S, from *asr2* (*a*ltered *s*ulfur *r*esponse) to *asr8,* focusing on the analysis of *asr2* and *asr3*. Sulfate ion content of *asr2-1* and *asr3-1* were higher in shoots than that of the wild-type. In *asr2-1*, expressions of genes induced by -S, such as *APR1*, and accumulation of *O*-acetyl-L-serine, a precurser of cysteine, were significantly higher in +S and lower in -S conditions compared to the wild-type. The causal gene was mapped to a 48 kb region on chromosome 5, in which the ferredoxin-dependent glutamate synthase gene (*GLU1*) was present. A nucleotide substitution of *GLU1* was found in *asr2-1*. Expressions of the -S-responsive genes and accumulations of amino acids in other lines with mutations in *GLU1* were similar to the case of *asr2-1*. These results indicate that the causal gene of *asr2* is *GLU1*, and that GLU1 is important for -S responses. GLU1 is known to be involved in nitrogen assimilation and photorespiration. Since the sulfur assimilation is considered to be coordinated with the nitrogen and carbon assimilation, it is not surprising we found that a defect in GLU1 caused altered responses to -S. However, the precise mechanism of these altered responses to -S is still unclear. Another mutant, *asr3-1*, was also analyzed and the causal gene was mapped to a 40 kb region on chromosome 1. In *asr3-1*, gene expressions and amino acid accumulations in response to -S were also different from that of the wild-type. Our results indicated a novel pathway regulating sulfur assimilation and responses.

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P32

Isolation and characterization of low-sulfur tolerant mutants of Arabidopsis

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Increasing sulfate utilization efficiency is an important issue for crop improvement. Little is known about the genetic determinants for sulfate utilization efficiency. Because of technical difficulties of low sulfur demand level by plants, no gain-of-function mutants with improved sulfate utilization efficiency have been reported to date. Here we report the isolation and characterization of two low-sulfur tolerant mutants, *sue3* (*s*ulfate *u*tilization *e*fficiency) and *sue4*, using a simple high-throughput genetic screen where a "sulfur-free" solid medium was devised to give the selection pressure necessary to suppress the growth of wildtype seedlings. Both mutants showed improved tolerance to low sulfur conditions and markedly increased root system, potentially having enhanced sulfate utilization efficiency. The mutant phenotype of both *sue3* and *sue4* was specific to sulfate deficiency and the mutants displayed enhanced tolerance to heavy metal and oxidative stress. Genetic analysis revealed that *sue3* was caused by a single recessive nuclear mutation while *sue4* by a single dominant nuclear mutation. Both mutations co-segregated with the selection marker on the T-DNA. Further analysis of the mutants will shed light on the genetic determinants of sulfur utilization efficiency.

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Gene expression analysis of transcription factors regulating methionine-derived glucosinolate biosynthesis

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PMG1, PMG2 and PMG3 are the transcription factors regulating methionine-derived glucosinolates (Met-GSL) biosynthesis [1,2,3]. Based on the glucosinolate profiles of the single knockout mutants of these genes, PMG1 is supposed to be a primary transcription factor and the others are supposed to be accessory transcription factors for Met-GSL biosynthesis. However, the expression levels of *PMGs* have not been fully discussed. In this study, we analyzed the amounts of three *PMGs* transcripts in the knockout lines of *PMG1, PMG2* and *PMG3*. Expression level of *PMG2* and *PMG3* was reduced to about 20% and 10%, respectively, in *pmg1*. On the other hand, *PMG1* expression level was reduced to about 60% in *pmg3*. *pmg2* did not affect the expression of *PMG1*. These results showed an interaction of *PMGs* at the expression level, where *PMG1* seems to have a regulating/superior function. To understand the function of PMG2 and PMG3, the expression level of both genes was evaluated using *pmg1* treated with methyl jasmonate (MeJA), a potent inducer of certain plant defense responses. As a result, the induction of *PMG2* and *PMG3* by MeJA was observed in *pmg1* as well as in wild-type, although the expression levels of both genes was lower in *pmg1*. In addition, the expression pattern of each *PMG* under sulfur- and nitrogen-stress conditions was different. Our working model of regulatory mechanism on Met-GSL biosynthesis will be discussed.

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Interaction of MYB and bHLH transcription factors in regulation of glucosinolate biosynthesis

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The transcription factor MYB51 is known as a positive regulator of indolic glucosinolates which act as plant defence compounds, allelochemicals and as cancer preventive phytochemicals in the human diet. Since it is known from other transcriptional regulators that they often act in concert with other gene regulators we were wanted to find out if there is also a regulatory network involved in the biosynthesis of indolic glucosinolates. An interaction between MYB- and bHLH-transcription factors is for example reported in many cases.

A yeast-2-hybrid screen indeed reveals a bHLH-transcription factor as a possible interaction partner for the MYB51. The *in vivo* interaction of MYB51 and the bHLH protein was confirmed by transient expression of split-YFP-constructs in tobacco leaves. Promotor-GUS studies displayed that both transcripts occur to large extent in the same tissues. An interaction of the gene products, as seen by protein interaction assays, is therefore possible *in planta*. The elucidation of the role of this protein-protein interaction in regulation of indolic glucosinolates is in progress.

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Investigating roles of genes induced by a short-term sulfur deficit: localization of UP9-UP9 interactions within tobacco cells

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It is well known that plants can adjust their metabolism in response to sulfur deficiency conditions, but still many questions concerning the regulatory mechanisms of this phenomenon remain unanswered. Analyzes of genes regulated by S-deficiency stress encoding proteins of unknown function is one of the way that could help to answer some of these questions.

One of these genes is *UP9* identified as strongly up-regulated at the transcript level in the 2-day starved tobacco plants using SSH method [1]. Phenotypical and biochemical analysis of transgenic plants with increased and silenced expression of UP9 suggest its essential role in a proper response of tobacco plants to sulfur starvation. The UP9 family includes at least five genes in tobacco but, so far, only two of them were identified as induced by sulfur deficit. Both encode 117aa products that contain three hypothetical domains: nuclear localization signal, phosphorylation site and coiled coil region. The coiled-coil domain is probably responsible for protein dimerization, which in turn could be involved in regulation of protein localization, interactions and function.

It has been known from the previous experiments performed in our laboratory, such as yeast 2-hybrid and "pull-down" assay in denaturing conditions that UP9 is able to form dimers. To confirm and possibly localize the interactions within the plant cells *in vivo* the FRET technique was used. The UP9 dimerization was shown in the leaves of the two-week old tobacco transformants containing UP9-CFP and UP9-YFP fusion proteins. We have observed a dimer signal mostly in nucleus, therefore we hypothesize that UP9 dimers are present only in this compartment and the dimerization could be an important regulatory mechanism of UP9 function. However, this hypothesis need to be confirmed using different methods.

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P36

Investigating roles of genes induced by a short-term sulfur deficit: preliminary characteristics of UP15 protein

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In many geographical regions sulfur (S) is a limiting factor in crop production and plants are exposed to S deficit stress. There are several factors responsible for such situation, for example decreased atmospheric pollution and usage of fertilizers free from S. Recently, the importance of a sufficient supply of S has become apparent. Several laboratories started intensive studies at the regulatory aspects, adaptation, tolerance and metabolic pathways in plants exposed to S deficit.

Our work is focused on one of tobacco genes encoding protein of unknown function. The *UP15* gene was identified as strongly upregulated during S deficit using suppression subtractive hybridization method [1]. The strong regulation of the expression of *UP15* by sulfate availability was confirmed using northern blots. The highest level of *UP15* transcript was observed in young and mature leaves. *UP15* cDNA and its predicted protein product was further investigated in this study. No close homologues of *UP15* could be identified in *Arabidopsis thaliana*. UP15 is a small protein containing a Gly-and His-rich regions and a potential nuclear localization sequence (NLS). It is probably located in nucleus, however, *in silico* analysis demonstrates that its localisation in chloroplast is also possible. In order to investigate the UP15 function we decided to use yeast 2-hybrid system to identify plant proteins that interact with this protein *in vivo*. The cDNA library was prepared from tobacco plants grown in the conditions of two days S deficit and several interacting proteins were identified. Results of this approach will be shown.

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Control of S assimilation in onion (*Allium cepa* L.)

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In onion (*Allium cepa* L.), a sulfur accumulating species, the reductive sulfur assimilation pathway, in common with other higher plants, begins with the activation of SO_4^{2-} by ATP sulfurylase to form 5'-adenylylsulfate (APS). Two further enzymes, APS reductase (APR) and sulfite reductase (SiR) reduce APS to produce sulfide, which is then incorporated into cysteine by the enzyme complex cysteine synthase. Also in common with other plant species, the transcription of APR is induced by low S supply suggesting a key control point at this part of the pathway. In part support of this, some evidence from *in vitro* studies has shown that recombinant APR and ATPS from onion can form a protein complex [1]. However, closer examination of the regulation of the pathway in onion, in response to S supply, has shown that while the transcription of APR and CS genes is induced in seedlings in response to low S supply, induction of enzyme activity does not occur until the bulbing (the high S demand stage) [2]. One mechanism for the separation of transcriptional and translational changes is post-translational modification of the gene products. In this poster, we present some preliminary evidence that evaluates whether some of the enzymes in the S-assimilation pathway are targets of phosphorylation.

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High resolution gene expression analysis of the sulfur-dependent transcriptome in *Arabidopsis thaliana*

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Sulfur is an elemental component of life. In plants, sulfur is not only involved in fundamental redox processes, but also necessary to cope with biotic and abiotic stress. Although much is known about expression of genes and the enzymatic steps of sulfate uptake and reductive sulfate assimilation in *Arabidopsis thaliana*, only little is known about the regulation of sulfur metabolism related transcriptome at higher resolution than shoot and root organs.
To investigate the regulation of sulfur metabolism related genes at the expression level, a custom-made gene array was designed. This array was performed for high specificity consisting of 50mer oligonucleotides for 920 gene probes. The combination of genes aims at sulfur-related processes including markers for primary metabolism, nutrition, redox relations and plant defense. Genes were selected for primary and secondary sulfur metabolism, nitrogen, phosphorus and carbon nutrition, mineral ion membrane transport, amino acid transport, redox regulation and pathogen defense. The selectivity and sensitivity of this array combines with cost effectivity for serial investigations as compared larger arrays that nearly cover whole genomes. Fine mapping of spatial distribution of sulfur-related gene expression will be possible as well as kinetics of expression profiles following stress treatments.

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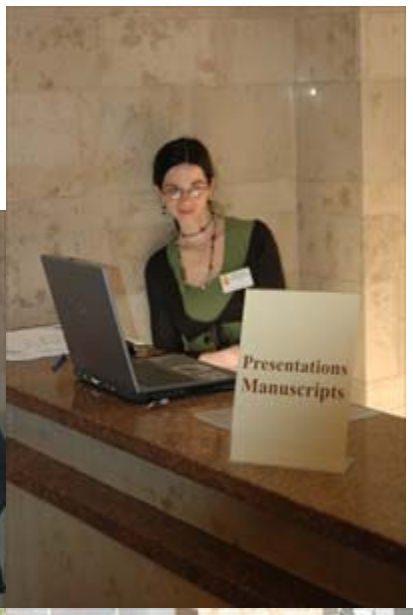
Photos



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