Hydrogen peroxide strongly accumulated after 2 days of dark-treatment in the leaves and activities of dehydroascorbate reductase, glutathione reductase and catalase were decreased, whereas ascorbate peroxidase showed increased activities during dark-induced senescence. Ascorbate levels were unchanged during senescence, while glutathione content strongly decreased in all subcellular compartments 1 day after the beginning of darkness in all investigated plants. The increase of H_2O_2 correlated temporally with the reduction of glutathione contents, since H_2O_2 contents started to increase only after 2 days of treatment. Ascorbate and glutathione play a crucial role during senescence. Beside its role as antioxidant, the content and subcellular distribution of ascorbate and glutathione were shown to have regulatory function on ROS accumulation, gene expression and redox signaling. The rapid decrease of glutathione contents in all subcellular compartments by dark-incubation of individual leaves could serve as senescence-inducing signal, regulating senescence, via creating an oxidative burst by an increase of H_2O_2 contents, which is needed for senescence-progression.

Thus, we can conclude that the compartment specific decrease of glutathione rather than ascorbate contents contributes to the accumulation of H_2O_2, which is a well-known signal for triggering senescence.

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15) Cadmium sensitivity of pad2-1 and vtc2-1 mutants is correlated to lower subcellular glutathione rather than ascorbate contents

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Toxic levels of cadmium (Cd) can cause alterations of the chloroplast ultrastructure, disturbs the synthesis of chlorophyll and carotenoids, leads to CO_2 deficiency due to stomatal closure and can cause several other metabolic disturbances. Indirectly Cd can cause oxidative stress and can lead to the accumulation of reactive oxygen species (ROS). Antioxidants such as ascorbate and glutathione can mitigate the effects of ROS by detoxifying them through the ascorbate-glutathione-cycle. Additionally, glutathione has the ability to bind to Cd directly and serves as a precursor of phytochelatins, which forms complexes with Cd and are then deposited in vacuoles. The aim of the study was to analyse the compartment specific distribution of ascorbate and glutathione over a time period of 14 days in Cd treated (50 µM and 100 µM) Arabidopsis Col-0 plants. To obtain a deeper insight into how possible limitations of ascorbate and glutathione contents affect the defense of plants against Cd we additionally analysed the importance of subcellular ascorbate and glutathione contents in mutants deficient in ascorbate and glutathione (*vtc2-1* and *pad2-1*, respectively). For this purpose we used a technique that visualizes glutathione and ascorbate in all cell compartments simultaneously in one experiment, which is based on immunogold-cytohistochemistry and computer-supported transmission electron microscopy.

In general, glutathione contents in wildtype plants followed recently proposed antioxidative stress models after treatment with 100 µM Cd in all cell compartments. Glutathione contents decreased during an initial alarming phase after 12 h (up to −84%), increased during a resistance phase between 48 h until 7d (up to 165%), and decreased during an exhaustion phase after 14 days of Cd-treatment (up to −45%). Compared to the wildtype both mutants showed strongly reduced glutathione contents (up to −90%) over the whole experiment after 100 µM Cd treatment. While ascorbate levels in wildtype plants and *vtc2-1* mutants followed the above described stress model after 100 µM Cd treatment, they were strongly reduced in the *pad2-1* mutant (up to −86%) throughout the experiment. After the exposure to 100 µM Cd both mutants showed earlier and stronger symptoms (96 h after treatment) like chlorosis and necrosis than the wildtype, which showed first symptoms 7 days after the treatment. 50 µM Cd treatment induced elevated glutathione contents in wildtype plants (up to 152%), decreased glutathione levels in the *pad2-1* mutant (up to −86%), whereas the *vtc2-1* mutant showed a typical bell shaped stress response curve over the whole experiment. Ascorbate contents after treatment with 50 µM Cd followed the above described antioxidative stress model in each plant. Symptom development remained absent in wildtype plants but both mutants showed first symptoms 7d after 50 µM Cd treatment. Although symptom development in the mutants appeared at the same time-point after Cd treatment the *pad2-1* mutant showed always stronger symptoms and larger necrotic areas than the *vtc2-1* mutant.

Wildtype plants showed a typical stress response curve with an immediate decrease in glutathione and ascorbate contents in all cell compartments, which indicates an excessive demand for antioxidants immediately after the exposure to 100 µM Cd. Such a reaction of plants seems likely as Cd has a high affinity to thiol groups and would bind to reduce glutathione in the cytosol after entering the cells. Additionally, glutathione is used for the production of phytochelatins, which are also involved in the detoxification of Cd. Considering that Cd enters the cytosol first and is then transported into the vacuoles it is interesting that both antioxidants decreased in all other cell compartments at similar rates. Thus, glutathione and ascorbate are either withdrawn from other cell compartments or transported from the origin of synthesis to the other cell compartments as they are used for Cd and ROS detoxification in the cytosol. The results also revealed that both mutants showed higher sensitivity to Cd treatment than the wildtype because of an earlier and stronger development of Cd-induced symptoms. The appearance of these symptoms is correlated with a strong decrease in glutathione contents in most cell compartments at these time-points and this indicates that low glutathione levels in the mutants were unable to protect plants against the toxic effects of Cd. The *pad2-1* mutant, a glutathione-deficient mutant which contains up to 90% less glutathione than wildtype plants, showed stronger symptom development than the *vtc2-1* mutant. This is another indication that Cd sensitivity is linked to low glutathione concentrations rather than changes in ascorbate contents.

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16) Metal toxicity and sulfur metabolism in Chinese cabbage

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Copper and zinc are essential nutrients for the plants; however, they become rapidly phytotoxic at elevated concentrations in the root environment. Exposure of Chinese cabbage to elevated Cu^{2+} (≥ 2 µM) or Zn2 + concentrations (≥ 5 µM) resulted in leaf chlorosis and subsequently in a loss of photosynthetic activity and a strongly reduced biomass production of both root and shoot. Cu is a redox-active metal, which has the potential to accelerate the formation of reactive oxygen species in plant tissue. However in Chinese cabbage, the Cu-induced chlorosis was most likely not due to the formation of reactive oxygen species but the consequence of a negatively affected chloroplast development. UV-A+B radiation and elevated Cu^{2+} concentrations had negative synergistic effects on biomass production, pigment content on the quantum yield of photosystem II.

The uptake and metabolism of sulfur and nitrogen were differentially affected at elevated Cu^{2+} or Zn^{2+} concentrations. Both Cu^{2+} and Zn^{2+} exposure resulted in an increased sulfate uptake by the roots and in enhanced total sulfur content of the shoot, which could be ascribed partially to an accumulation of sulfate. Moreover, exposure resulted in a strongly enhanced level of water-soluble non-protein thiols in the root, which only partially could be ascribed to a metal-induced synthesis of phytochelatins. The nitrate uptake by the root was decreased upon Cu^{2+} or Zn^{2+} exposure, demonstrating the absence of a mutual regulation of the uptake of sulfate and nitrate. There was no direct relation between the sulfur metabolite levels viz. total sulfur, sulfate and water-soluble non-protein thiols and the expression and activity of the sulfate transporters and the expression of APS reductase at elevated Cu and Zn concentrations. Apparently, the presumed signal transduction pathway involved in their regulation appeared to be overruled or bypassed at high tissue Cu and Zn levels. It is doubtful that the Cu^{2+} or Zn^{2+}-induced effects on the uptake and metabolism of sulfate have any adaptive significance in the detoxification of these metals in Chinese cabbage.

Elevated Zn^{2+} concentrations in the root environment did not only disturb the uptake, distribution and assimilation of sulfate, it also affected the uptake and metabolism of nitrate in Chinese cabbage. The uptake of nitrate appeared to be closely linked to the growth rate of the plant, even at toxic Zn levels. The total N content was strongly decreased in the shoot at toxic Zn concentrations. The decrease in total N in the shoot could only partly be ascribed to a decrease in nitrate content. In the root, however, the total N content remained unaffected at elevated Zn^{2+} concentrations.

17) Using H^+-selective microelectrodes to study the adaptive response of plant roots to sulfate deficiency

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The knowledge about the response of the sulfate uptake system of plants to sulfate deficiency on the level of gene expression has increased a lot during the last decades. However, there is still a need for a deeper understanding of the bio- and electrochemical processes involved in plant adaptation to sulfate deficiency. Ion-selective microelectrodes are a useful tool to answer questions in various fields of plant physiology, e.g. salt stress, calcium signaling, growth regulation and defense responses to pathogens. In plant nutrition ion-selective microelectrodes have been applied to study e.g. the uptake preference of plants for different nitrogen sources, and the uptake of calcium and potassium into wheat seeds. However, determination of sulfate uptake by plant roots using ion-selective microelectrodes was not carried out until now, probably also due to the lack of a liquid ion exchanger that is selective enough for sulfate over other anions. Sulfate is taken up by plant sulfate transporters via sulfate/proton-symporters and in the current case study the activity of sulfate transporters of plant roots was measured by using the non-invasive *Microelectrode Ion Flux Estimation* method (MIFE). Roots of seedlings of Chinese cabbage which were grown under sufficient nutrient conditions were compared with roots that had been sulfur deprived with the result that the latter showed a much higher proton influx in response to addition of $MgSO_4$. This indicated that the activity of the sulfate uptake system could be determined adequately by measuring proton fluxes using the MIFE technique. Sulfate-deficient plants acidified their rhizosphere via proton efflux, while sulfate sufficient plants did not. Furthermore the extent of the response to $MgSO_4$ addition to roots of sulfate-deficient plants correlated strongly with the root surface pH prior addition. This indicated that acidification might be an important component of the adaption to sulfate deficiency, as known for other nutrients such as phosphate and iron. This component might be an explanation for the discrepancies between the expression levels of sulfate transporters and the actual sulfate uptake capacity that are sometimes observed under sulfate deficiency. The involvement of the plasma membrane located ATPase in this adaptation will be further examined. Using MIFE, it was also possible to record root profiles of the sulfate import along the first seven millimeters from the root tip. Preliminary results suggest that there is no sulfate uptake taking place in the first 3.5 mm from the tip, but in the mature zone of the root. H^+-selective electrodes show promise as tool to further investigate adaptive responses of plant roots to sulfate deficiency, other than solely an up-regulation of the sulfate transporter genes.

18) Metabolic coordination of sulfur metabolism in *Brassica napus* by clock-controlled genes

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Experiments done with *Arabidopsis* plants so far, such as results from microarrays, indicate that some key enzymes in sulfur metabolism are regulated diurnally and/or by the circadian clock. Understanding metabolic changes in plants and determining output-related function of clock genes could help in elucidating circadian-clock mechanisms underlying plant growth and development. The sulfur requirement of the plants fluctuate during plant development and vary between species differing in sulfur need for growth and the potential sink capacity of secondary sulfur compounds.

Several sulfur-containing metabolites are involved in pathogen defense mechanisms and are suggested as compounds to enhance defense (SED = sulfur enhanced defense). We analyzed whether in addition to genes involved in sulfur metabolism also the levels of sulfur-containing metabolites such as cysteine and glutathione were clock regulated. They could be released rhythmically to the apoplastic fluid or via the stomata and defend the plants against attacking pathogens.

Oilseed rape (canola) is one of the most important agricultural crop plants for oil and declared as renewable resource. Two different *Brassica napus* L. (oilseed rape) lines were analyzed on the transcriptomic and metabolomic level in light/dark and