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 free-running conditions. Different sulfur fertilization was applied to analyze the effects on pathogenesis-related compounds. Results from these experiments could help to optimize the use of fertilizer and if applicable reduce the amount of fungicides/pesticides.

19) The effect of the continuous light in combination with sulfur deprivation on the chlorophyll levels and carotenoids in young maize leaves

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Light fulfills two significant roles in plant growth. At first, light drives photosynthesis by providing energy and second it is perceived by several photoreceptors, thus activating signal pathways. Continuous light changes plant physiology by affecting both roles, thus creating difficulties in identifying the factors that are responsible for injuries under such treatment. As far as we know, the effect of continuous light in combination with plant's nutritional status or nutritional deficiency on its physiology is poorly studied. In particular, there are no references with regard to sulfur deficiency. Towards this direction, the responses of *Zea mays* plants to light environment in combination with nutrition were studied in four treatments; C: normal photoperiod & complete nutrient solution, Cc: continuous light & complete nutrient solution, −S: normal photoperiod & nutrient solution without sulfur, −Sc: continuous light & nutrient solution without sulfur. Plants were grown for seven days under normal photoperiod condition and then the treatment was applied for 3 weeks. The photon flux density was not modified during this period. The effect of the above mentioned cases on growth was monitored via fresh mass measurement, whilst the corresponding effect on the photosystems antennas was followed by determining the extractable levels of Chl *a*, Chl *b* and carotenoids from leaf lamina or sheath, by means of dimethyl sulfoxide. Our results showed that the treatments affected the time of organ appearance as well as their presence itself. The ratio of Chl *a/b* as well as the ratio of carotenoids to total chlorophyll proved to be useful response indicators to each treatment. The dynamic of adjustments presented by the sheaths (Sh) were different than the corresponding ones presented by the laminas (L).

Under continuous light and complete nutrition, the influence focused on L_4, L_5, L_6, whilst L_7 did not occur. Sheaths appearance was not affected except for Sh_5. Injuries due to this condition focused on youngest leaf from L_4 onwards and Sh_4 onwards. The lamina overall average of Chl a/b ratio was 4.1 (an increase by 13.9%), whilst in sheaths it was 2.7 (decreased by 6.9%). The laminas average Car:Chl ratio was 2.1 (decreased by 4.5%), whilst in sheaths it was 3.1 (increased by 10.7%).

A two days delay was observed in laminas L_5, L_6, L_7 and sheaths Sh_2, Sh_3 during the treatment of sulfur deficiency under normal photoperiod. No injuries were caused in laminas. The average Chl *a/b* ratio of the laminas was 3.8 (increased by 5.6%), whilst the average one in sheaths was 2.6 (decreased by 10.4%). In laminas, the average of Car:Chl ratio was 2.5 (increased by 13.6%), whilst in sheaths the corresponding average was 2.9 (increased by 3.6%).

With regard to treatment with continuous light combined with sulfur deficiency, the appearance of organs took place at the same time as in control plants, with the exception of L_7 and Sh_2. This fact indicates that the deficiency eliminated the effect of continuous light. Aging and collapsing was observed at the oldest organs L_0, L_1, Sh_0, Sh_1. In laminas, the average value of Chl *a/b* ratio was 3.8 (increased by 5.6%), whilst in sheaths the average was 2.7 (decreased by 6.9%). At the end of the experiment, in laminas the average of Car:Chl ratio was 2.7 (increased by 22.7%), whilst in sheaths the corresponding average was 2.8 (as in control plants).

20) Aerenchyma formation in maize leaves during sulfate deprivation

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Aerenchyma is the term given to plant tissues containing enlarged gas spaces exceeding those commonly found as intracellular spaces. So far, aerenchyma formation under nutrient deficiencies and especially under nitrogen- or phosphorus- or sulfur deficiency has been reported only in the adventitious roots of maize by lysis of cortical cells. Seven-day-old maize plants were grown in a hydroponics setup for nineteen days under sulfate deprivation against plants grown under full nutrition and samplings were taken at day 17 and 26 from sowing (day 10 and day 19 of the deprivation respectively). Samples from the fresh laminas of the 2nd leaves were fixed and embedded in paraffin. Sections were received with microtome from the top, middle and base of the lamina and stained with safranin-fast green. The dry mass and water amount, the sulfate and total sulfur contents and the specific surface area of the 2nd leaf lamina and the transpiration rate of the plant were determined.

Under the circumstances we observed the presence of enlarged spaces in this lamina of the S-deprived plants, a fact that to our knowledge has not been reported so far. More specific, on the 10th day under the deprivation, the cross sections from the top of the 2nd leaf lamina of the S-deprived plants, revealed larger substomatal chambers compared to the control plants under full nutrition. In the middle of the S-deprived plants lamina-enlarged spaces appeared among the vascular bundles probably caused by lysis of mesophyll cells. These enlarged spaces stretched from the upper to the lower epidermis or between the stoma and the epidermis with equal frequency of appearance, whilst they appeared fewer times between the upper and the lower stoma. The percentage of the aerenchyma in relation to the total cross section area reached 4.9%. On the base of this lamina enlarge gas spaces appeared too, however to a very small extent, since the percentage of the aerenchymatous area was 0.3% of the total area of the section. On this day, the 2nd leaf of the S-deprived plants contained a significantly lesser amounts of sulfate, organic sulfur and total sulfur by 74%, 38% and 48% respectively compared to control plants. The S-deprived lamina's dry mass and water amounts were as in control. The specific surface area of the lamina (dry mass per lamina surface area) of the S-deprived plants was less by 19% compared to control plants. The S-deprived plants presented a larger transpiration rate by 28% than the control plants. These data indicate that on the tenth day of deprivation, aerenchyma may be formed in maize leaves in response to sulfur deficiency following the described pattern between the vascular bundles.

On the 19th day under the deprivation, such enlarged spaces appeared only in the middle of the lamina of the S-deprived plants 2nd leaf and the percentage of this aerenchyma reached just the 0.7% of the total cross section area. On this day, the 2nd leaf of the S-deprived plants presented less amounts of organic