Abiotic stress occurs when plants are exposed to high concentrations of sulfur dioxide (SO_2) and its derivative sulfite. Physiological studies suggested sulfite oxidase (SO) as one important component of SO_2 detoxification. However, it is unknown to which extend plant fumigation with SO_2 triggers a specific transcriptional response. To address this question, we compared Arabidopsis wildtype (WT) and SO knock-out lines (SO-KO) facing the impact of 600 nL L^{-1} SO_2 using RNAseq to quantify absolute transcript abundances. These transcriptome data were correlated to sulfur metabolism related enzyme activities and metabolites obtained from the same samples in a physiological study. SO-KO plants showed first symptoms of leaf injury after fumigation that were not detectable in fumigated WT plants. Analogously, SO-KO exhibited remarkable and broad regulative responses at the mRNA level, especially in transcripts related to sulfur metabolism enzymes but also of those related to stress response and senescence. Our data provide evidence for a highly specific co-regulation between SO and sulfur related enzymes like APS reductase. Moreover, we could show that beside of SO a new player comes into business – apoplastic peroxidase (PRX). PRXs known from work of Pfanz and colleagues in the early 1990ies are able to detoxify sulfite. We could show that under SO_2-fumigation the mRNA of *prxcb* is 5fold up-regulated. This result implies that sulfite detoxification is of exceptional importance so that the plants have evolved a complex network of mechanisms and enzymes for controlling its level including regulation of stomata, generation of enzymes like SO and PRX for direct oxidation and use of the sulfur assimilation pathway for reductive detoxification.

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

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

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

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

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

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

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

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

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Excess light conditions represent a potential danger to the plant as it can lead to the accumulation of reactive oxygen species (ROS) in chloroplasts by overstraining the electron transport chain during photosynthesis and of hydrogen peroxide (H_2O_2) in peroxisomes as a result of glycolate oxidation in the photorespiratory pathway. If not detoxified ROS can leak into the

cytosol and other cell compartments where they are capable of oxidizing membrane components and proteins and can cause the degradation of nucleic acids, lipids, pigments, membranes, proteins, RNA, and DNA, causing mutation and eventually cell death. Ascorbate and glutathione are the most important antioxidants that are able to detoxify ROS. They occur in all cell compartments at high concentrations. Whereas an increase in ascorbate contents during high light conditions was commonly found with biochemical methods, unchanged levels of glutathione were observed during such conditions, indicating a more important role for ascorbate in the protection against high light stress. Nevertheless, all of these studies investigated ascorbate and glutathione levels in whole leaves and therefore the antioxidative defense situation on the subcellular level remained unclear. The aim of this study was to investigate subcellular changes in ascorbate and glutathione contents in Arabidopsis Col-0 plants during the exposure to high light (150 versus 700 μmol m^{-2} s^{-1}) in order to clarify the dynamic compartment specific protection of these key antioxidants against ROS produced during high light conditions.

On the subcellular level both ascorbate and glutathione were strongly increased in most cell compartments during high light conditions, as observed by transmission electron microscopy after immunohistochemical detection of ascorbate and glutathione. A very strong increase was detected in chloroplasts (104% for ascorbate and 190% for glutathione) after the exposure to light conditions of 700 μMol m^{-2} s^{-1} for 2 weeks. This data highlights the importance of both ascorbate and glutathione in the antioxidative protection against oxidative stress induced in this cell compartment during high light conditions. Both ascorbate and glutathione could also be detected inside the lumen of thylakoids of chloroplasts exposed to high light stress. This observation is interesting in respect to non-photochemical quenching which decreases the formation of ROS by dissipation of excess absorbed light as heat. One important mechanism for non-photochemical quenching is the formation of zeaxanthin to violaxanthin that is catalysed by the enzyme violaxanthin de-epoxidase. This enzyme is located inside the thylakoid lumen and uses ascorbic acid as a reductant. Additionally, ascorbate can be used as an alternative electron donor by photosystem II and I, which is especially important in situation of stress when the linear electron transport is impaired. Thus, the detection of ascorbate in the thylakoid lumen of plants exposed to high light conditions and the general increase inside the stroma of both antioxidants highlights the importance of high ascorbate and glutathione contents for the compartment specific protection of chloroplasts during high light conditions.

One very important aspect is the massive increase of ascorbate specific labeling in vacuoles during high light exposure (up to 395%). It has been proposed recently that ascorbate plays an important role in the detoxification of H_2O_2 that diffuses into vacuoles especially during environmental stress situations. In this cell compartment ascorbate helps to reduce phenoxyl radicals (created by oxidation of phenols by H_2O_2) and is oxidized to mono- and dehydroascorbic acid, which is then transported into the cytosol for reduction to ascorbic acid. Thus, it can be concluded that, in contrast to glutathione which did not occur in vacuoles, ascorbate plays important roles in vacuoles during excess light conditions most probably by direct or indirect detoxification of H_2O_2.

Summing up, we can conclude that in Arabidopsis wildtype plants the accumulation of ascorbate and glutathione especially in chloroplasts is an important mechanism to protect plants against ROS produced in this cell compartments during high light stress. Additionally, the accumulation of ascorbate in vacuoles of wildtype plants indicates an important role of this antioxidant in vacuoles for the detoxification of H_2O_2 leaking from peroxisomes and chloroplasts into the cytosol and vacuoles.

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14) The role of antioxidant metabolism during dark-induced senescence in Arabidopsis

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Senescence is a developmental program and highly regulated by specific genes. Senescing cells undergo drastic metabolic changes and organized degradation of cell structures. Antioxidants were shown to play an important role during senescence and many senescence-related genes were shown to be induced by reactive oxygen species (ROS). The equilibrium between the production and the scavenging of ROS is regulated by antioxidants as well as antioxidative enzymes. The aim of this study was to investigate the compartment specific importance of ascorbate and glutathione and related enzymes during dark induced senescence in *Arabidopsis thaliana* Col-0.

In this study senescence was induced by darkening of individual leaves, which has been shown to be an appropriated method to synchronize the senescence process in leaves. To further characterize the impact of the antioxidants ascorbate and glutathione in the regulation of senescence, the *Arabidopsis* mutants *vtc2-1* and *pad2-1*, deficient in ascorbate and glutathione, respectively were used in this study.

After initiating leaf senescence by dark treatment a yellowing of the leaves could be observed which correlated well with a decrease in pigment content of the leaves. Light microscopic investigations revealed a decrease of the number of chloroplasts per cell during senescence in wildtype plants and both mutant lines, already after 2 days of dark-treatment. Together with the decreased number of chloroplasts per cell, an overall reduction of size could be observed during senescence. A reduction of the size and number of chloroplasts has been observed in many plants species during senescence. In this study, prominent structures, diverse deposits and small vesicles, engulfed by single or double membranes could be observed in the cells during dark-induced senescence. These structures established throughout senescence processes in *Arabidopsis* wildtype, and the mutant lines *pad2-1* and *vtc2-1* to the same extent. Different compartments, deposits and vesicles were shown to be involved in degradation processes of cellular components.

A precise analysis of chloroplasts inner structure by transmission electron microscopy (TEM) revealed a massive degradation of senescing chloroplasts. When leaves were subjected to dark treatment starch grains were degraded within the first 2 days of treatment. Chloroplasts under control conditions contained an organized network of photosynthetic thylakoid membranes, starch grains and a number of small plastoglobuli droplets. During senescence thylakoid membrane system was found to decrease in size, which correlated with an increase in plastoglobuli area. Plastoglobuli are known to contain lipids derived from the degradation of thylakoids as well as chlorophyll, carotenoids, photosynthetic proteins. The degradation of thylakoid membranes is accompanied by the downregulation of photosynthesis during senescence. The degradation of chloroplasts was shown to be one of the most significant and earliest events during senescence and its degradation is a major source of nutrients which are recycled during leaf senescence.