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.

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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