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

Barbara Koffler, Lisa Polanschütz, Bernd Zechmann
University of Graz, Institute of Plant Sciences, Graz, Austria
E-Mail: barbaraeva.koffler@edu.uni-graz.at

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

Luit J. De Kok[1], C. Elisabeth E. Stuiver[1], Freek S. Posthumus[1], Muhammad Shahbaz[1,2], Saroj Parmar[3] and Malcolm J. Hawkesford[3]
[1] Laboratory of Plant Physiology, Centre for Ecological and Evolutionary Studies, University of Groningen, Groningen, The Netherlands
[2] Biology Department and Program in Molecular Plant Biology, Colorado State University, Fort Collins, USA
[3] Plant Biology and Crop Science Department, Rothamsted Research, Plant Biology and Crop Science Department, Harpenden, Hertfordshire, UK
E-Mail: l.j.de.kok@rug.nl