Subcellular quantification of glutathione and its precursors during pathogen attack in plants

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Glutathione is an antioxidant and involved in the detoxification of reactive oxygen species, which are commonly formed during environmental stress situations, and lead to the destruction of biological membranes, proteins, RNA and DNA leading to mutation, cancer and eventually cell death. Changes in glutathione contents are therefore commonly used as stress markers during investigations in most fields of plant sciences. Nevertheless, as glutathione metabolism involves highly compartment specific pathways possible limitations in the ability of glutathione to protect the plant against stress situations can only be detected if glutathione contents are analyzed at the subcellular level.

For this purpose an immunogold cytohistochemical approach was developed and adapted to different plant material in order to detect and quantify subcellular glutathione and its precursors with computer-supported transmission electron microscopy [1, 2]. These studies showed that the distribution of glutathione is similar in different plant species (*Arabidopsis thaliana*, *Cucurbita pepo*, *Nicotiana tabacum*, *Beta vulgaris* Fig.1). Surprisingly, plastids, which have been described before as major site of glutathione accumulation, contained the lowest amounts of glutathione. Highest glutathione contents were always found in mitochondria, while glutathione-labeling in the cytosol, nuclei and peroxisomes was intermediate. No glutathione was detected in vacuoles and the apoplast (Fig.1). The accuracy of the glutathione-labeling method was supported by different observations. First, pre-adsorption of the anti-glutathione antisera with glutathione reduced the density of the gold particles to background levels. Second, the overall glutathione-labeling density was reduced by about 90% in leaves of the glutathione-deficient *Arabidopsis* mutant *pad2-1* and increased in transgenic plants with enhanced glutathione accumulation. These experiments also showed that mitochondria maintained high and stable glutathione levels during situations of glutathione deficiency, which indicates that glutathione in mitochondria plays an important role in plants for cell survival.

Further studies showed that glutathione synthesis during pathogen attack is limited by the availability of glutathione precursors. Whereas a tolerant pumpkin species (which showed a stronger increase in glutathione contents than the susceptible one) contained increased levels of glutathione precursors during virus infection the susceptible one showed generally decreased levels. These results also indicated that cysteine might be the limiting factor for glutathione synthesis during compatible virus-infection. By artificial elevation of cysteine a strong increase in glutathione contents and subsequently a suppression of symptom development during compatible virus infection in Styrian oil pumpkin plants could be observed, which were correlated with decreased amounts of viral particles within leaves and roots [3].

Summing up, the development and application of these novel methods revealed that glutathione precursors (especially cysteine) limit the operation of glutathione metabolism during pathogen attack. The modification (increase) of cysteine contents in plants during

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pathogen attack resulted in a strong increase in glutathione contents and subsequently in a higher stress tolerance during virus infection in Styrian oil pumkin plants. Thus these studies and methods can now be used for the development of new defense strategies for agricultural use in the future, and can protect farmers from possible crop losses induced by environmental stress situations in the future.

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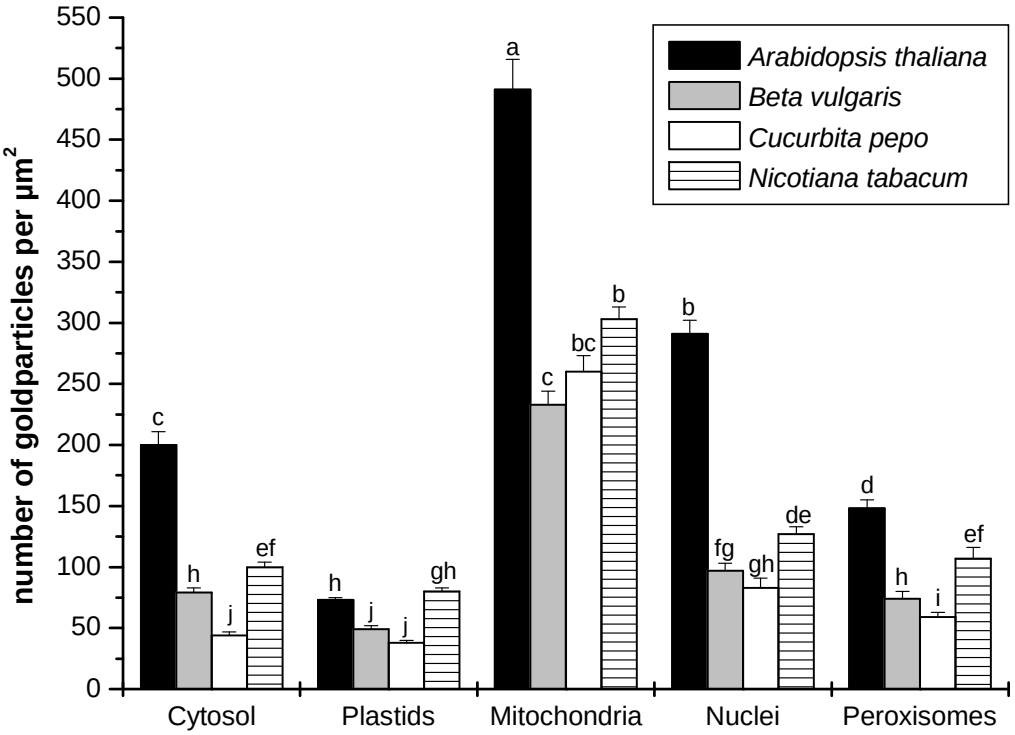

Figure 1. Graph shows glutathione labeling densities within different cell compartments of leaves of four different plant species. Values are means with standard errors and document the amount of gold particles per μm^2. Significant differences between the samples are indicated by different lowercase letters; samples, which are significantly different from each other, have no letter in common. $P < 0.05$ was regarded significant analyzed by the Kruskal-Wallis test, followed by post hoc comparison according to Conover. $n > 20$ for peroxisomes and $n > 60$ for all other cell structures.

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