Subcellular distribution of glutathione in the gametophyte

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Glutathione is an important antioxidant and redox buffer in plants. Despite its crucial roles in plant metabolism and defense, its roles in the gametophyte are largely unexplored. Recently, we demonstrated that glutathione synthesis is essential for pollen germination in vitro. In this study, we extend these results and focus on the subcellular distribution of glutathione in pollen grains and compare it to the situation in the sporophyte. Glutathione was equally distributed within mitochondria, plastids, nuclei and the cytosol in the gametophyte—in contrast to youngest fully-developed leaves and root tips of the sporophyte, where glutathione was highest in the mitochondria, followed by nuclei, cytosol, peroxisomes and plastids in decreasing concentration. Glutathione was not detected in vacuoles. We can conclude that glutathione synthesis is essential for pollen germination in vitro and that the subcellular distribution of glutathione in the gametophyte differs significantly from the sporophyte.

Importance of Glutathione in Plants

The tripeptide glutathione ($\gamma$-glutamylcysteinylglycine) has many important roles in plant metabolism and defense. It detoxifies reactive oxygen and nitrogen species (ROS/RNS) and is also involved in the modulation of gene expression, redox signaling, and in the regulation of enzymatic activities (extensively reviewed in ref. 1 and 2). Within plants, glutathione occurs in its reduced (GSH, thiol form) and oxidized forms (GSSG, glutathione disulphide). In non-stressed plants glutathione occurs mainly as GSH, whereas during situations of oxidative stress large amounts of GSSG can be formed.4-3 Synthesis of glutathione takes place in two ATP-dependent steps. In the first step, cysteine is linked together with glutamate to form the intermediate product $\gamma$-glutamylcysteine ($\gamma$-EC). In the second step, glycine is added to form the final product glutathione.3,4 Even though the roles of glutathione are well-defined and investigated in the sporophyte, very little information is available about its importance in the gametophyte. A major reason is that collecting sufficient pollen to measure glutathione in the gametophyte is technically challenging using the current technologies. With biochemical methods, a high quantity of pollen grains needs to be harvested at similar developmental stage to get representative results, which is quite difficult considering the small size and quantity of pollen present in the anthers of Arabidopsis plants.5 Additionally, it remains unclear how the different harvesting procedures affect glutathione contents, which are very sensitive to environmental stress. The main limitation of conventional light microscopic methods (e.g., staining of glutathione with fluorescent dyes) is that the fluorescent dyes do not label glutathione in all cell compartments.3,7 This, accompanied by the native autofluorescence of the exine walls, make it difficult to evaluate whether the obtained fluorescence represents the real glutathione content in the pollen grains. Recently we have adapted a cytohistochemical approach using Arabidopsis gametophytes in order to study the subcellular distribution of glutathione by quantitative immunogold labeling.5 The main advantage of this method is that it allows the study of the subcellular distribution of glutathione in all cell compartments of single pollen grains simultaneously with a high level of resolution in one experiment. In a recent study, we were able to demonstrate that glutathione occurs in the gametophyte and that its synthesis is essential for pollen germination in vitro.6 These results are especially interesting as bioinformatic data cast doubt on the importance of glutathione metabolism in the male gametophyte, as both enzymes (GSH1 and GSH2) involved in glutathione synthesis are transcribed at negligible levels in pollen and sperm cells.6,10 (Fig. 1). In juvenile leaves and root tips of the sporophyte, much higher transcription levels of GSH1 and GSH2 are found (Fig. 1), which correlate with much higher glutathione contents in these organs in adult Arabidopsis plants when compared with pollen grains (Fig. 2A). From the data presented in the previous study, we concluded that sufficient translated product of GSH1 and GSH2 must be present in the gametophyte and that glutathione is essential for pollen germination in vitro during development and germination—most probably due to the important roles of glutathione as an antioxidant and as an important regulator and modulator for enzyme activities and gene expression, respectively.8 In this study, we extend

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the highest concentration of label in leaves and roots of the sporophyte was found in mitochondria followed by nuclei, the cytosol, peroxisomes and plastids. In plants, high levels of glutathione accumulation in vacuoles and chloroplasts.

Glutathione was found to be distributed essentially equally in mitochondria, plastids, nuclei and the cytosol containing similar amounts of glutathione. These data are significantly different from the situation found in the sporophyte, where glutathione levels are quite substantially higher (over 30-fold higher in the sporophyte compared with the gametophyte) when compared with the gametophyte (Fig. 2A). The highest concentration of label in leaves and roots of the sporophyte was found in mitochondria followed by nuclei, the cytosol, peroxisomes and plastids. In plants, high levels of glutathione in mitochondria are important for proper plant development and low levels of glutathione can lead to severe growth phenotype.12 Higher glutathione levels in vacuoles of leaves were only achieved when internal glutathione contents shifted more toward the oxidized form or reached higher levels than found in nature, e.g., during sulfur treatment.16 This was not the case during this study when pollen grains of both the wild-type and the glutathione-deficient mutant pad2-1 were germinated on 3 mM GSH. Even though glutathione increased in pollen grains over 2-fold in wild-type and over 12-fold in the pad2-1 mutant, no glutathione appeared to be localized in vacuoles (Fig. 3C). Thus it seems that glutathione in vacuoles plays a negligible role in the gametophyte.

In conclusion, this and the previous study clearly demonstrated that glutathione is equally distributed throughout the gametophyte, despite compartment-specific differences in the sporophyte, and that glutathione synthesis is essential for pollen germination in vitro.

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References


**Figure 2.** Statistical analysis of gold particle density in leaves, roots and pollen grains. Graphs show the labeling density of glutathione within different cell compartments of the youngest fully developed leaves, root tips and pollen grains of Arabidopsis Col-0 (A), and the pad2-1 mutant (B). Values are means with standard errors, which document the concentration of gold particles per μm². 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, using n > 20 for peroxisomes and n > 60 for all other cell structures.

**Figure 3.** Transmission electron micrographs showing the subcellular distribution of glutathione in Col-0 and pad2-1 pollen grains. Gold particles bound to glutathione were evenly distributed in plastids (P), mitochondria (M), and the cytosol, but were absent in vacuoles (V) of pollen grains obtained from Arabidopsis thaliana accession Col-0 (A) and the pad2-1 mutant (B and C). Pollen grains were grown on solidified pollen germination medium for 4 h (A and B) without GSH (control) and (C) with 3 mM GSH prior to fixation. Bars = 0.5 μm.