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Sub-cellular distribution of glutathione in an Arabidopsis mutant (*vtc1*) deficient in ascorbate

Nieves Fernandez-García, Maria C. Martí, Ana Jimenez, Francisca Sevilla, Enrique Olmos*

Department of Stress Biology and Plant Pathology, CEBAS-CSIC, Campus de Espinardo, P.O. Box 164, 30100 Espinardo-Murcia, Spain

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Summary

Glutathione and ascorbate are considered the major redox buffers in plant cells. They are implicated in many reactions at the sub-cellular level. However, information about the location and quantification of glutathione in the different sub-cellular compartments is very scarce and it has been obtained mainly using organelle purification and chemical quantification. We have used a glutathione antibody to immunolabel and quantify the total glutathione in leaves from wild-type *Arabidopsis thaliana* (Col-0) and an *A. thaliana* mutant (*vtc1*) deficient in ascorbate. Spectrophotometrical quantification has shown that this mutant has a higher content of glutathione during plant development compared with Col-0 [Pavet V, Olmos E, Kiddle G, Mowla S, Kumar S, Antoniw J, et al. Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiology 2005;139:1291—03]. We have observed, using immunolabelling techniques, that mitochondria showed the highest density of glutathione labelling in both Col-0 and *vtc*1 plants during all developmental stages and that the lowest density occurred in the chloroplasts, for both lines. However, the distribution of glutathione in the different sub-cellular compartments indicates that the chloroplasts contain about 62—75% of the total cellular glutathione and that the mitochondria represent the second greatest pool, with about 15—25% of the total cellular glutathione.

It has been observed previously that the *vtc1* mutant exhibits an induction of cell death and disease resistance in the face of pathogen attack. The differing distributions and concentrations of glutathione in the mitochondria of wild-type *A. thaliana* and the *vtc1* mutant is discussed.

Abbreviations: AGB, 1-amino-4-guanidobutane; GSH, reduced glutathione; GSSG, oxidised glutathione; PBS, phosphate-buffered saline; PCD, programmed cell death; ROS, reactive oxygen species.

*Corresponding author. Tel.: +34 968 39 63 36; fax: +34 968 39 62 13.
E-mail address: eolmos@cebas.csic.es (E. Olmos).

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Introduction

Ascorbate and glutathione are considered the major redox buffers of plant cells (Noctor and Foyer, 1998). They are involved in redox homeostasis and redox-signalling events (Foyer and Noctor, 2005). Moreover, both these antioxidant compounds control reactive oxygen species (ROS) accumulation and interact with numerous cellular components. Previous studies using Arabidopsis mutants that are deficient in ascorbate (*vtc1* and *vtc2*) showed that low ascorbate abundance leads to microlesions and enhanced basal resistance against pathogen infection caused by *Pseudomonas syringae* (Pavet et al., 2005). In these mutants, low ascorbate triggers programmed cell death (PCD). These microlesions were observed even in the young rosettes (4–6 weeks) as small areas of collapsed cells, but after 8 weeks these areas of dead cells were strongly expanded (Pavet et al., 2005). The *vtc1* and *vtc2* mutants showed a delayed development phenotype compared to the wild-type, both mutants were smaller at equivalent stages in development than wild-type plants (Pavet et al., 2005). The *vtc1* mutant was isolated in the ozone sensitivity screen, showing a defect in GDP-mannose pyrophosphorilase and around 30% of the leaf ascorbate present in wild-type (Conklin et al., 2000). These ascorbate-deficient mutants showed a higher content of glutathione compared with the wild-type (Col-0) during plant development. In these mutants, low ascorbate triggers PCD. The mitochondria play a key role in maintaining the redox homeostasis in cooperation with other organelles, mainly the chloroplasts (Noctor et al., 2007). Therefore, the accumulation of ROS in the mitochondria under biotic and abiotic stress could trigger PCD.

Little is known about the sub-cellular distribution of glutathione in plants. Quantitative analyses have been developed using purified organelles (Jimenez et al., 1997; Palma et al., 2006). This technique presents some problems. Glutathione is a highly diffusible compound that can move out from the organelles during the purification process. Moreover, contamination from other organelles, such as nuclei, chloroplasts or cytosol, should be considered also (Palma et al., 2006). For these reasons, organelle purification should be done using accurate methodology as described in the bibliography (Jimenez et al., 1997, 1998; Palma et al., 2006).

In recent years, new methods have been used to quantify and localise glutathione at the intracellular level (Muller et al., 2005). These methods are based on microscopy techniques using monochlorobimane fluorescence or on the antibody developed against glutathione. Monochlorobimane labelling of glutathione permits its sub-cellular location, using laser confocal techniques for *in vivo* samples (Fricker and Meyer, 2001; Meyer et al., 2001). However, monochlorobimane cannot be used to quantify the glutathione concentration in the different sub-cellular organelles (Muller et al., 2005).

On the other hand, glutathione seems to be fixed *in situ* by cross-linking with the surrounding proteins, using glutaraldehyde as fixative. Therefore, the combination of glutaraldehyde and paraformaldehyde can be used efficiently to fix the glutathione *in situ* and maintain an acceptable fixation of the tissues for immunolabelling. This method has shown itself to be efficient with regard to immunolocalisation of glutathione in the different sub-cellular compartments of plant cells (Muller et al., 2004). However, this technique has been scarcely used in the bibliography. Recently, Zechmann et al. (2008) have immunolocated glutathione in *pad2-1* Arabidopsis mutant which is deficient in glutathione, showing a decrease of 90% of the total glutathione but mitochondria contents was remained very similar to wild-type.

The aim of the present work was to study the sub-cellular distribution of glutathione in an Arabidopsis mutant (*vtc1*) deficient in ascorbate. We want to show that the major compartment in glutathione location is the chloroplast using the immunolabelling technique described above.

Material and methods

Plant material

Wild-type (Col-0) and *vtc1* seeds were germinated in compost, at 4 °C in the dark, for 7 d and then transferred to pots containing the same compost in controlled-environment chambers maintained at a day/night temperature of 20 °C, a relative humidity of 70% and a 10-h photoperiod (200 μmol m^{-2} s^{-1}). Leaf material was harvested for resin embedding at three different stages of development of leaf: young leaf (2 weeks old plants leaves, at this point, are under expansion), fully expanded leaf (6 weeks old plants leaves, at this point, are totally expanded but plants are not bolting) and mature leaf (10 weeks old plants leaves, at this point, are wild-type have bolted but not *vtc1*). For this experiment, at least four different plants were used. For 2 weeks old plants samples were taken from the second leaf and for 6 and 10 weeks old plants samples were taken from the fully expanded leaves. We have also taken