LM/TEM IMMUNOLOCALIZATION OF LOW MOLECULAR WEIGHT ANTIOXIDANTS IN OLIVE POLLEN DURING POLLEN DEVELOPMENT AND GERMINATION

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Both ascorbate and glutathione represent low molecular weight multifunctional metabolites, considered essential for plant development and growth. They play crucial roles as antioxidants against biotic and abiotic stresses, also contributing to redox signalling, modulation of gene expression and the regulation of diverse enzyme activities. Ascorbate homeostasis occurs through the activity of a complex network of enzymes involved in its synthesis and in both ascorbate oxidation and regeneration. This last takes place in a large proportion with the concurrence of the NADPH generated during the oxidation of glutathione. Therefore, the levels of both chemicals are tightly correlated throughout the so-called ascorbate-glutathione pathway [1-3].

The physiological roles of both glutathione and ascorbic acid in plants are beginning to be characterized, in many cases with the help of mutants defective in several key enzymes [3]. Moreover, the compartmentalization of these low molecular weight components has been described in several plant sources, either by TEM immunolocalization or by derivatization to fluorescent components and fluorescence microscopy localization in the case of glutathione [2, 3, 4]. These studies report the wide presence of glutathione and ascorbate in many different subcellular localizations including plastids, mitochondria, cytosol, the vacuole and even the nucleus from the leaf mesophyll, epidermal, root cells and other tissues. Such *in situ* immunolocalizations rely mainly in the use of specific antibodies to these low molecular compounds, some of them commercially available. Labelling quantification methods performed after the immunocytochemical procedures have also allowed correlating ultrastructural observations with those results obtained after whole tissue quantification by chemical methods.

In this work we have used two commercial antibodies to glutathione and ascorbic acid, respectively in order to determine tissue and subcellular localization of these chemicals in the olive pollen throughout pollen development and *in vitro* germination. These studies have been carried out at both LM (with the concurs of Alexa-488 fluorescence-labelled secondary antibodies), and TEM. The results showed that both molecules are present in the microsporocites and other tissues of the anther (including the tapetum) along the whole developmental process, and in the pollen grain and the elongating pollen tube

with a broad distribution. This includes organelles (pastids, mitochondria), the cytosol and (in the case of glutathione), the apertural regions of the pollen grain and the nuclei with a remarkable intensity. This localization suggests a key role of these components in pollen metabolism and physiology, as has been determined already in somatic tissues. Moreover, the likely involvement of glutathione in the control of the cell cycle in pollen is discussed.

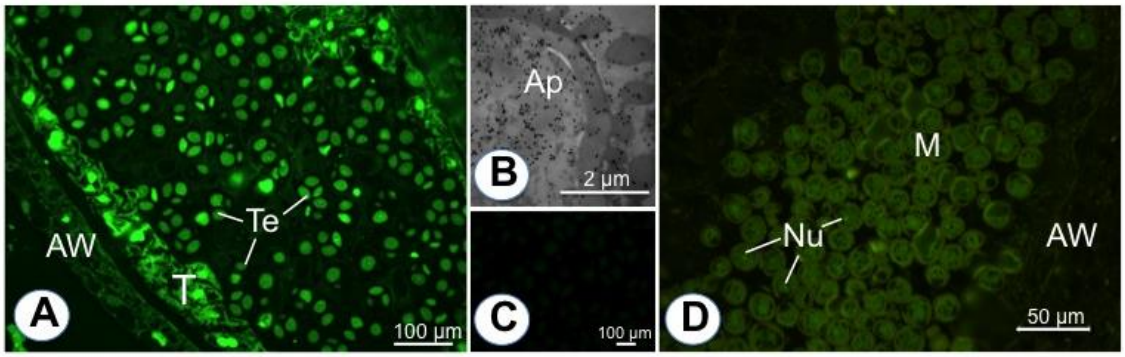

Figure 1: Immunolocalization of glutathione (A, B) and ascorbate (D) during olive pollen development. A) Fluorescence microscopy localization of glutathione in the olive anther at the tetrad stage. B) TEM immunolocalization of glutathione in the apertural region of a mature pollen grain. D) Fluorescence microscopy localization of ascorbate in the olive anther at the microspore stage. C) negative control for fluorescence microscopy prepared by omitting the primary antibody. Ap: aperture region, AW: anther wall, M: microspores, T: tapetum, Te: tetrads,

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