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Peroxisomal localization of CuZn superoxide dismutase in the male reproductive tissues of the olive tree

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Superoxide dismutases (SODs) are a class of antioxidant enzymes which catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, therefore controlling cellular levels of Reactive Oxygen Species (ROS). In the mature pollen grains of the olive tree, the presence of several forms of CuZn-SOD and the cytosolic localization of the enzyme have been described [1]. The present study was aimed to elucidate the adaptation of the oxidative metabolism to the changing conditions occurring during the course of olive pollen formation, hydration and pollen tube emergence and growth. We used a polyclonal antibody (raised against a KLH-linked synthetic peptide including a consensus sequence for CuZn-SODs in olive pollen) in immunocytochemical experiments carried out by Fluorescence (FM) and Transmission Electron Microcopy (TEM).

CuZn-SOD immunolocalization by FM revealed the presence of differences in the expression of the enzyme depending on the developmental stage and the analyzed reproductive tissue (Figure 1). In the anther tissues, the fluorescent signal became highly visible at the stage of tetrad, where most of the cells of the tetrad (particularly at their periphery) and the cells of the anther wall and the tapetum revealed green fluorescence. The fluorescent signal displayed an increase at the stages of "early microspore" and "mature pollen grain inside the anther", with an intense signal observed in the different anther wall layers, including the senescent tapetum. Both the microspores and the mature pollen grains showed fluorescence labeling localized in the cytosol and the microspore/pollen wall. A majority of the pollen grains presented a "spotty" labeling pattern, probably corresponding to peroxisomes. Although CuZn-SOD is considered as a characteristic matrix enzyme of peroxisomes in different tissues like oilseed cotyledons [2], the presence of the enzyme has not been previously associated to these organelles in olive pollen.

In order to dip into this possibility, we carried out TEM immunolocalization experiments by using the same antibody to olive CuZn-SOD (Figure 2). In addition to the labeling associated to the cytosol, the apertural region and the pollen wall (already described for olive pollen), intense labeling was detected in roundly-shaped structures with a slight electron-dense matrix, present in the cytoplasm of the vegetative cell. Confirmation of their nature as peroxisomes was accomplished by immunolocalization using an antibody reactive to catalase, a peroxisomal marker enzyme, prepared against a synthetic peptide designed using a consensus sequence of different plant catalases. Co-localization of both enzymes in these structures was also detected.

References

1. Alche *et al.*, Physiol. Plantarum 104: 772-776, 1998
2. Corpas *et al.*, New Phytol. 138: 307-314, 1998

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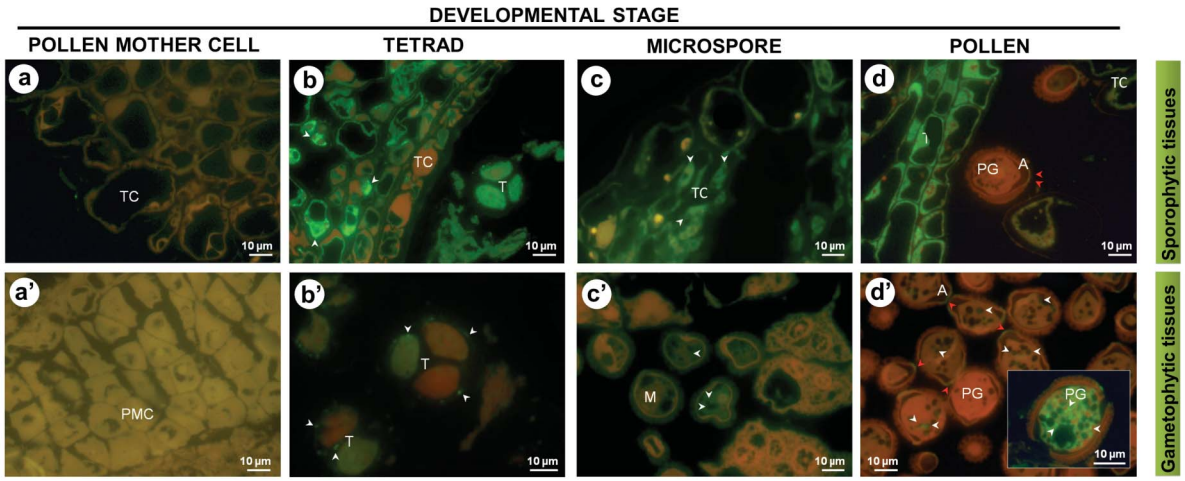

Figure 1. FM immunofluorescence localization of CuZn-SOD in the anther tissues throughout different developmental stages. Green fluorescence reveals the presence of the enzyme (Alexa fluor 488 secondary antibody). The red color is due to the autofluorescence. White arrowheads point to CuZn-SOD localization. Red arrowheads indicate signal in the apertural region of the mature pollen grains. Images were gathered with a digital camera attached to a Zeiss Axioplan Fluorescence Microscope using a standard FITC filter combination. A: aperture; M: microspore; PG: pollen grain; PMC: pollen mother cells; T: tetrad; TC: tapetal cells.

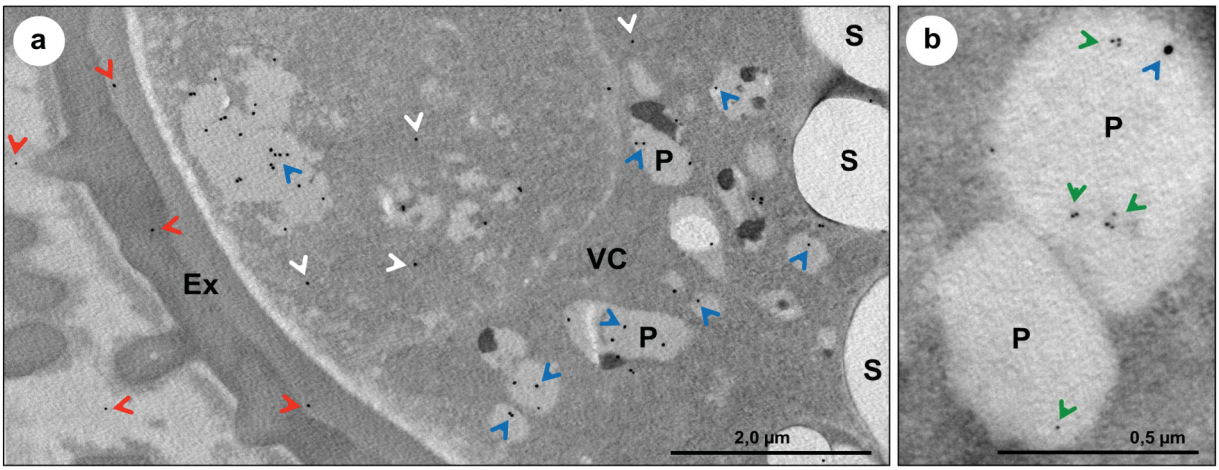

Figure 2. a: TEM immunolocalization of CuZn-SOD revealed its presence in the exine and the material adhered to this structure (red arrowheads). The enzyme is also localized in the cytosol (white arrowheads), and organelles of different shapes and sizes, frequently more or less rounded (blue arrowheads). b: co-localization of catalase (10 nm particles, yellow arrowheads) and CuZn-SOD (25 nm particles, blue arrowheads) in putative peroxisomes. Ex: exine; P: peroxisomes; S: starch; VC: vegetative cell cytoplasm.

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