ABSTRACTS OF THE JOINT MEETING OF THE SPANISH AND PORTUGUESE MICROSCOPY SOCIETIES

XXIV CONGRESS OF THE SPANISH MICROSCOPY SOCIETY
XLIV ANNUAL MEETING OF THE PORTUGUESE SOCIETY FOR MICROSCOPY

SEGOVIA, 16-19TH JULY 2009

IE University
Santa Cruz Convent
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SEGOVIA, SPAIN.

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3-D ANALYSIS OF THE LOCALIZATION OFPECTINS AND AGPs IN
THE OLIVE POLLEN GRAIN AND POLLEN TUBE.

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Different studies dealing with the immunolocalization of arabinogalactan proteins (AGPs) and pectins are allowing us to draw a picture of the dynamic of pollen tube wall synthesis in the olive during in vitro pollen germination [1, 2]. These studies use a panel of antibodies, which are able to recognize different types of epitopes.

Both the pollen and the pollen tube walls are complex structures. Although the immunocytochemical determinations performed on sections (either thin sections of embedded material or optical sections) permit detailed dissection of the signal, an overall, 3-D view of the localization often helps to acquire a better understanding of the dynamics of these components.

In this work, we have used olive pollen grains germinating in vitro, to assess the localization of both neutral pectins (by using the antibodies LM5 and LM6), and proteoglycans (by using the antibodies JIM13 and JIM14). The pollen cultures were slightly fixed chemically and incubated with the primary antibody. An anti mouse/rat IgG Alexa488-conjugated was used as the secondary antibody. Observations were performed in a Nikon C1 confocal laser scanning microscope equipped with an Ar-488 laser source. Z-stacks of the samples were obtained. The images of the optical sections recorded simultaneously both the signal of the antibody and the slight autofluorescence of the pollen exine and the pollen tube cytoplasm.

The files of the images were treated with the Imaris 4.0.6 software (Bitplane AG), in order to generate 3-D reconstructions of the labeled structures. Several visualization modes were assayed. Most images offered optimal resolution/3-D quality under the “Easy3D” shadow projection, which mimics a light source illuminating each channel in its channel colors and creates shadows on both the background and the data.

Using this imaging model, labeling was clearly identified as sharp, green-colored surfaces in the apertural regions of the pollen grains, the inner or outer layers of the pollen tube wall, the pollen tube apex etc., depending on the antibody used. The autofluorescence displayed by the pollen wall (including the apertural regions) and the cytoplasm of the pollen tubes appeared labeled in red, offering a good level of 3-D resolution too. Upper- and bottom-views of the 3-D reconstruction are also available.

The use of other 3-D imaging modes is being assayed, including the “surpass” mode (visualization of iso-surfaces, and an ortho-slicer displaying a slice of the dataset).

As mentioned above, all these modes of 3-D reconstruction offer excellent overall view of the growing pollen tubes, which contribute to a better interpretation of the localization of the labeling. They also improve the quality of images for presentation purposes.
This work was supported by research projects P06-AGR-01719 (Andalusian Regional Government) and BFU2008-00629 (Spanish MCyT).

References

Figure 1: immunolocalization of neutral pectins [(1→4)-β-D-gal]4 with the antibody LM5. Green labelling is localized in the active aperture. Comparison of the images obtained after Z-stack projection, and 3-D reconstruction and shadowing of the optical sections. Ap: aperture. PG: pollen grain. PT: pollen tube. Bars represent 10 μm.