

30

years of (e-) microscopy  
at the EEZ in images



30

years of (e<sup>-</sup>) microscopy  
at the EEZ in images

---

Edited by Antonio J. Castro and Juan de Dios Alché

## **30 years of (e<sup>-</sup>) microscopy at the EEZ in images**

**Editors: Antonio Jesús Castro López  
Juan de Dios Alché Ramírez**

**Published by the Plant Reproductive Biology Research Group  
Estación Experimental del Zaidín (CSIC)  
Profesor Albareda 1  
18008 Granada, Spain  
E-mail: [buzon@eez.csic.es](mailto:buzon@eez.csic.es)  
<http://www.eez.csic.es>**

**First published November, 2013  
Printed in Granada (Spain)**

**ISBN 10: 84-616-7277-1  
ISBN 13: 978-84-616-7277-6**

## Microscopy to study plant sexual reproduction

Juan de Dios Alché\*, Antonio Jesús Castro, M<sup>a</sup> Carmen Fernández, Juan David Rejón, Krzysztof Zienkiewicz, Sonia Morales, Mohamed M'rani, José Carlos Jiménez-López, Agnieszka Zienkiewicz, José Angel Traverso, Adoración Zafra, M<sup>a</sup> José Jiménez-Quesada, Estefanía García-Quirós, Concepción Martínez-Sierra and M<sup>a</sup> Isabel Rodríguez-García

Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain

\*corresponding author: juandedios.alche@eez.csic.es

- **Pollen development under an ultrastructural view: first TEM studies at the EEZ**

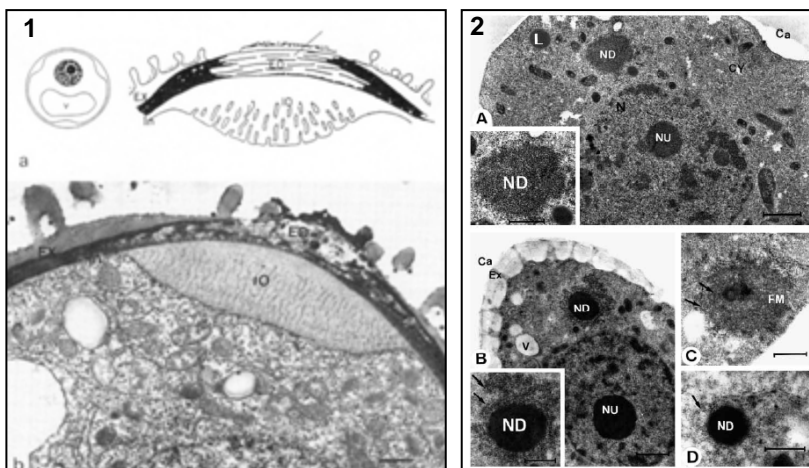


Figure 1. (A) Aperture formation in the olive pollen [Fernández and Rodríguez-García (1989) *New Phytol.* 111: 717]. (B) Cytochemical features of nucleoloids in olive microsporocytes [Alché *et al.* (1994) *J. Cell Sci.* 107: 621].

- **Bright field and fluorescence microscopy applied to the study of pollen development**

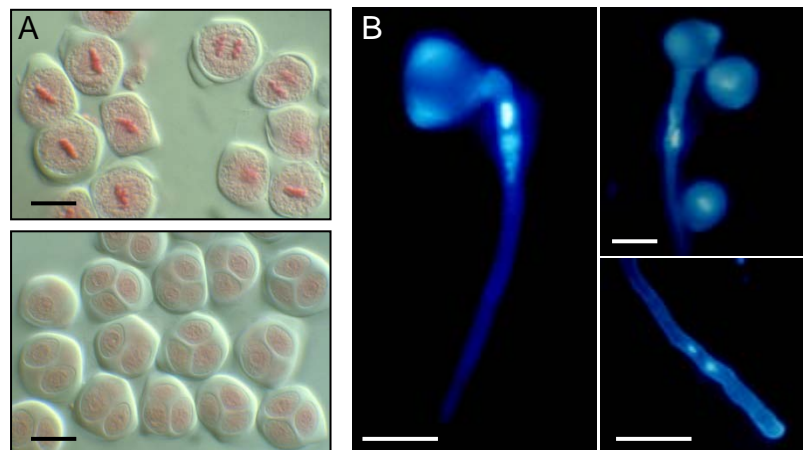


Figure 2. (A) Study of olive male meiosis by orcein staining and differential interference contrast (DIC) microscopy [Alché JD (1991) PhD thesis]. (B) Study of nuclear dynamics in the olive pollen tube by DAPI staining and fluorescence microscopy.

- **Scanning Electron Microscopy (SEM): surfing on the surface**

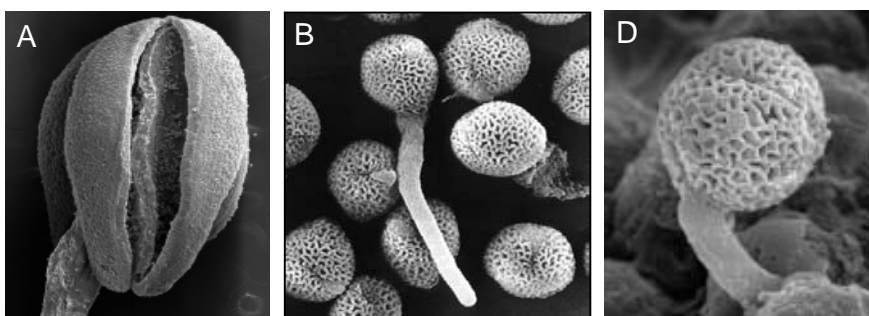


Figure 3. (A) SEM photomicrograph of an olive dehiscent anther. (B-C) SEM photomicrograph of olive pollen grains germinating *in vitro* (B) and *in vivo* through the stigmatic papillae (C).



• Cellular localization of biological molecules: what, where and when

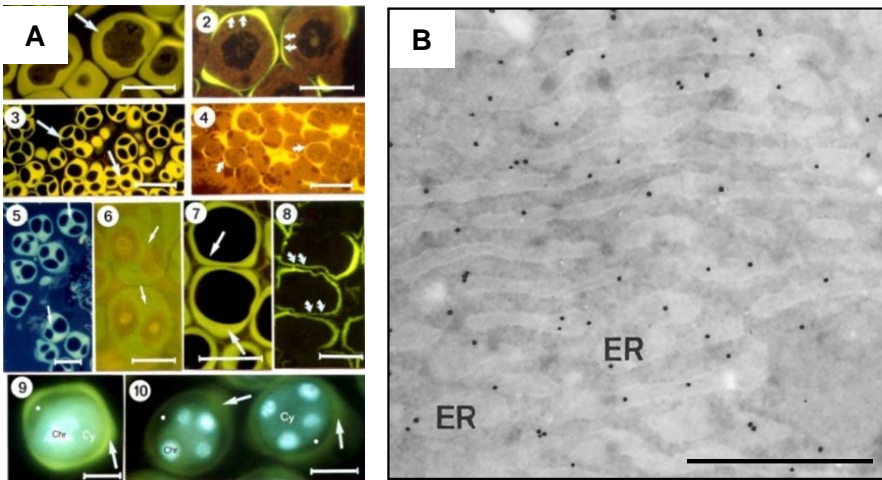


Figure 4. (A) Localization of callose by aniline blue in olive meiocytes [Alché and Rodríguez-García (1997) *Biotech. Histochem.* 72: 285]. (B) Immunolocalization of Ole e 1 protein in mature olive pollen grains. ER, endoplasmic reticulum [Rodríguez-García *et al.* (1995) *Planta* 196: 558].

• Confocal Laser Scanning Microscopy (CLSM): deep tissue imaging and 3-D reconstruction

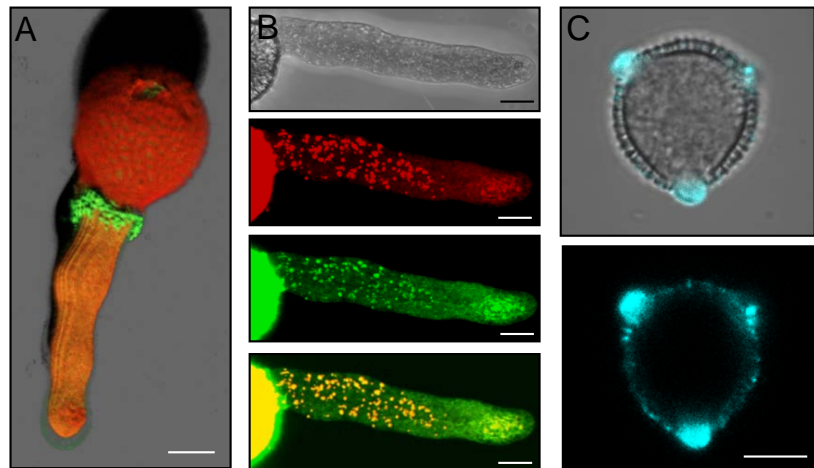


Figure 5. (A) Localization of galactans (green fluorescence) in germinating olive pollen [Castro *et al.* (2013) *Ann. Bot.* 112: 503]. (B) Co-localization of caleosin (green) and oil bodies (red) in olive pollen tubes [Zienkiewicz *et al.* (2010) *J. Exp. Bot.* 61: 1537]. (C) Localization of profilin in olive pollen [Morales *et al.* (2008) *J. Microsc.* 231: 332].

• Beyond structure: microscopy and function

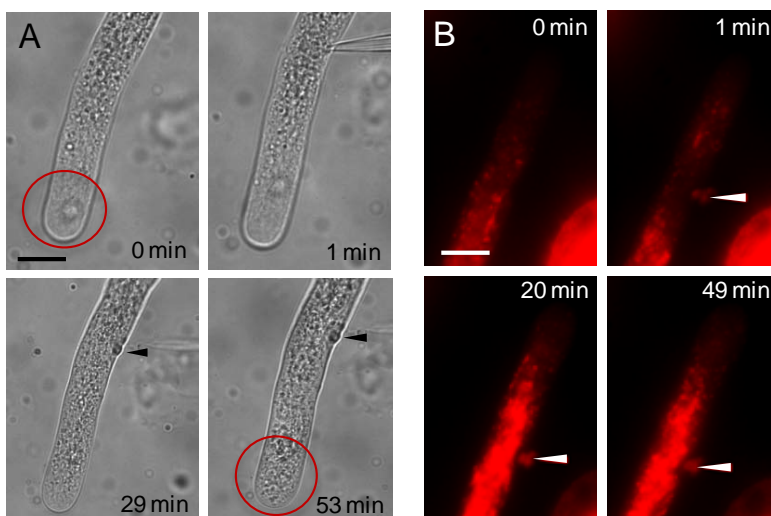


Figure 6. (A) Time course transmitting light microscopy analysis of pollen tube behaviour after antibody microinjection. After 1 h, the pollen tube growth stopped and the clear zone was not longer visible (red circle). (B) Time course fluorescence microscopy analyses of organelle distribution, motility and behavior after vital stain + antibody microinjection [Zienkiewicz *et al.* unpublished].

**Acknowledgements.** This work has been supported by numerous research projects. Currently active projects include EFDF con-funded grants BFU2011-22779 (Ministerio de Economía y Competitividad), P2010-CVI5767, P2010-AGR6274 and P2011-CVI7487 (Junta de Andalucía), and PEOPLE-PIOF-GA-2011-301550 (European Research Council).