

# 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, 16-19<sup>TH</sup> JULY 2009

IE University  
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**SEGOVIA, SPAIN.**



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## CLASSIFICATION AND DISCRIMINATION OF OLIVE CULTIVARS BY USING POLLEN MORPHOLOGICAL AND ULTRASTRUCTURAL FEATURES.

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Olive variety identification is crucial to safeguard the genetic patrimony of this species, as well as to fulfil the objectives of the breeding programmes and accomplish varietal authentication in plant nurseries. Up to date, different morphological parameters have been used to discriminate among cultivars (i.e. morphology of the endocarp, length, shape and size of leaves, inflorescences, fruit etc.). During the last decade, the use of molecular markers has been implemented for these same purposes, in order to improve both reliability and discriminative ability. The use of both types of markers is fully compatible and desirable [1].

This work describes pollen morphology and ultrastructure as an additional tool for the identification of olive varieties. Observations were carried on the following cultivars: Picudo, Lucio, Picual, Loaime, Hojiblanca and Manzanilla de Sevilla (Spanish varieties), Frantoio (Italian), Ayrourni (Lebanese), Chmlali and Chetoui (Tunisian).

For this purpose, dehiscent pollen grains were directly observed by transmitted light microscopy using a Zeiss Axioplan microscope in order to determine major morphological parameters (i.e. lengths of both longitudinal and equatorial diameters, grain areas...). For ultrastructural observations, pollen grains were processed according to standard procedures for scanning electron microscopy (chemical fixation, ethanol series dehydration, critical point and carbon and gold coating) [2] and transmission electron microscopy (chemical fixation, ethanol series dehydration, Epon embedding and sectioning) [3]. Observations were made in a LEO GEMINI-1530 SEM, and in a JEOL JEM-1011 TEM, respectively.

Multiple parameters were determined after morphometric analysis of the captured images using the iTEM (Soft Imaging System GmbH, 5.0 -build 1032-) software. Data were subjected to different statistical analysis, including ANOVA (Friedman) and multivariate analysis. Parameters like the lengths of both the equatorial and longitudinal diameters and the total surface were significantly different among pollens from diverse cultivars. Ultrastructural parameters involved in the exine ornamentation like width and height of the muri, and the area of orbiculi were also particularly cultivar-distinctive. Phylogenetic analysis can be constructed on the basis of the reported parameters.

Microscopy is thus revealing itself as an invaluable tool to discriminate the phylogenetic relationships among olive cultivars, which could be used alone, or in combination with other morphological and molecular approaches. Further developments of the methods used are discussed.

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### References

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- [2] Smyth D.R., Bowman, J.L. and Meyerowitz E.M. (1990). Plant Cell 2, 755-767.
- [3] Rodríguez-García, M.I. and Fernández, M.C. (1990). Plant Syst. Evol. 171, 221-231.

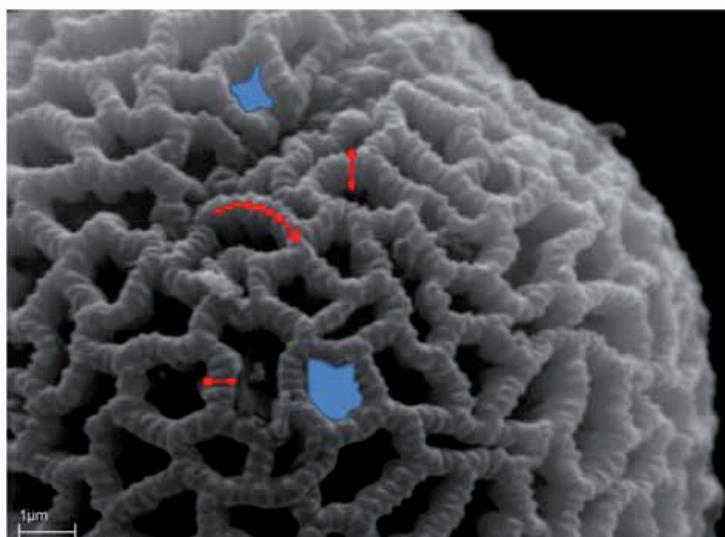


Figure 1: SEM image of an olive pollen grain showing some of the parameters measured.

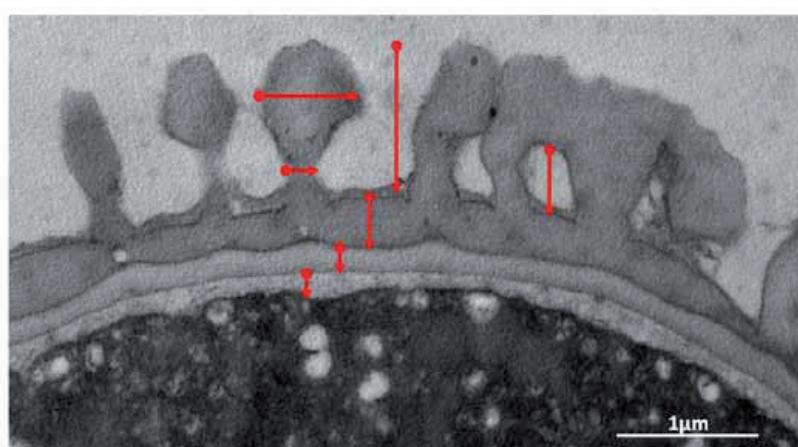


Figure 2: TEM image of an olive pollen grain showing details of the pollen wall and some of the parameters measured.