


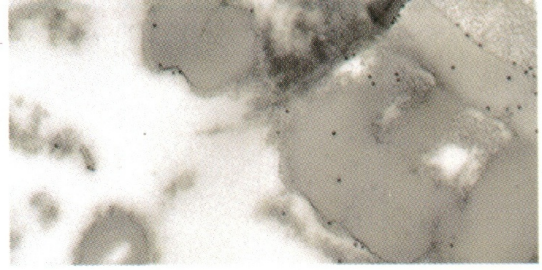

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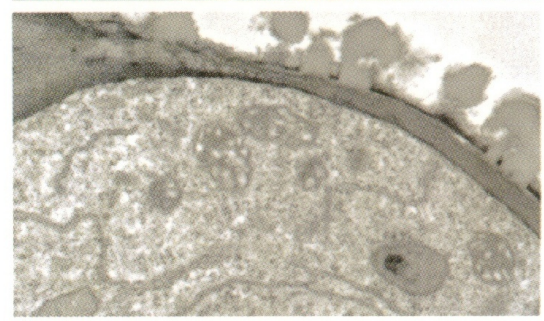

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An evaluation of methods assessing pollen quality in the olive

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Fruit formation in plants is the consequence of the successful meeting between male and female gametes, which leads to double fertilization. Pollen quality is a key factor in seed setting success [1]. This parameter is also important for monitoring the vigour of pollen batches that are used for pollination in breeding programs, as well as for determining which cultivars are more suitable as pollinizers in a given crop. Pollen quality largely depends on intrinsic (i.e. genetic origin) and environmental (e.g. temperature, humidity, etc.) factors. The aims of this work were: 1) to evaluate several methods assessing pollen quality in the olive, and 2) to analyze the variations in terms of pollen quality among different olive cultivars.

Mature pollen grains were collected from olive trees of different cultivars. Both pollen viability and germinability were used as indicators of pollen quality. The criteria to evaluate viability were the presence of enzyme activity and/or an intact plasmalemma in the pollen grain. Thus, pollen was stained with either fluorescein diacetate (FDA) [2] or trypan blue [3] followed by visual counting using a microscope. Alternatively, pollen was stained with FDA followed by counting of fluorescent grains in a flow cytometer. Pollen germinability was tested on the basis of the ability of pollen to germinate in vitro after three hours of incubation in the germinating medium and to form a pollen tube at least as long as pollen diameter. For this purpose, pollen was germinated in vitro according to [4]. Then, germinated and non-germinated grains were either counted using a microscope or discriminated by size in a flow cytometer. In all cases, both pollen viability and germinability rates were presented as percentages.

We found significant differences in terms of pollen viability and pollen germinability among the 11 olive cultivars tested ($p=0.05$, test K of Kruskal-Wallis), independently of the method used. Viability values varied between 20 and 70%, while germinability rates were considerably lower and ranged from 5 to 35%. However, a positive correlation was observed between both parameters after performing the Spearman´s rank correlation test. Comparison of viability and germinability values obtained by flow cytometry and visual counting methods showed significant differences in both parameters for some of the cultivars analyzed ($p= 0.05$, test U de Mann-Whitney). Large populations of germinated and non-germinated pollen grains (N= 10,000 per sample) were easily discriminated by size and counted in 10-15 minutes in a flow cytometer. This fact makes the subsequent statistics more robust and reliable than in the case of traditional counting-based methods in which only a few hundred pollen grains (usually 200-300 grains) are computed.

[1] Knox, R.B. 1984. Enclycl Plant Physiol 17: 508–608.

[2] Heslop-Harrison, J. and Heslop-Harrison, Y. (1970) Evaluation of pollen viability by enzymatically induced fluorescence; Intracellular hydrolysis of fluorescein diacetate. Stain Tech. 45: 115–120.

[3] Grato, P.L.; Pompeu, G.B.; Capaldi, F.R.; Vitorello, V. A.; Lea, P.J. and Azevedo; R. A. (2008) Antioxidant response of Nicotiana tabacum cv. Bright Yellow 2 cells to cadmium and nickel stress. Plant Cell Tiss Org Cult 94: 73–83.

[4] M'rani-Alaoui, M. (2000) Estudio a nivel celular de la germinación del polen, emisión y elongación del tubo polínico en el olivo (Olea europaea L.). PhD Thesis, University of Granada, Spain.

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