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Morphology and discrimination features of pollen from Italian olive cultivars (*Olea europaea* L.)

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Abstract

Pollen morphology of 14 cultivars of *Olea europaea* subsp. *europaea* var. *europaea* was analysed in order to discriminate main pollen types. The cultivars were selected from the most spread and early flowering crops grown in Italy. Morphometric parameters were observed on acetolysed pollen by means of light microscopy and scanning electron microscopy. Polar axis (*P*), equatorial diameter (*E*), *P/E* ratio, maximum distance between colpi in mesocolpium, distance between the apices of two colpi, exine thickness, maximum length of lumina in mesocolpium and in apocolpium, and exine reticulum thickness in mesocolpium have been measured. According to *P* and *E*, the 14 olive cultivars of this study can be divided into the three groups of small (*P*: 21.75 µm, *E*: 22.55 µm; 'Manna' and 'Tonda di Cagliari'), large (*P*: 25.1 µm, *E*: 26.1 µm; 'Pescarese' and 'Rotondella di Sanza') and medium size (*P*: 23.49 µm, *E*: 24.54 µm, 'Carolea', 'Grossa di Cassano', 'Giarraffa', 'Nocellara messinese', 'Nocellara del Belice', 'Santagatese', 'Intosso', 'Maiatica di Ferrandina', 'Nostrale di Fiano Romano', 'Santa Caterina'). Maximum length of lumina and exine thickness are useful parameters for further distinction of olive pollen groups, since these parameters are able to provide a specific pollen profile for each cultivar.

Keywords: pollen, olive tree, Mediterranean Basin, morphometry, exine patterns

Olea europaea L. is one of the most important crop trees marking the development of cultural landscapes in the Mediterranean Basin since the last three millennia (Mercuri 2014). The olive tree belongs to the Oleaceae family that includes about 25 genera and 600 species (Johnson 1957; Rohwer 1996; Wallander & Albert 2000; Green 2004; Besnard et al. 2009). *Olea* includes 30 species, among which *Olea europaea* is of major agricultural importance (Bracci et al. 2011).

Six geographically separated subspecies (Green 2002) constitute the *Olea europaea* complex (Hannachi et al. 2009; Bracci et al. 2011): subsp. *laperrine*

(Batt. et Trab.) Ciferri, present in the mountains from central Sahara to northeast Sudan (Ozenda 2004; Besnard et al. 2007); subsp. *cuspidata* (Wall. ex G. Don) Ciferri, spread from South Africa to southern Egypt and from Arabia to northern India and south-west China (Boulos 2000; Green 2002); subsp. *guanchica* P.Vargas, J.Hess, Muñoz Garm. et Kadereit, in the Canary Islands; subsp. *maroccana* (Greuter et Burdet) P.Vargas, J.Hess, Muñoz Garm. et Kadereit, in south-western Morocco; subsp. *cerasiformis* (Webb et Berth.) Kundel et Sunding, endemic in Madeira (Green 2002); and subsp. *europaea* spread all over the Mediterranean Basin (Figure 1).

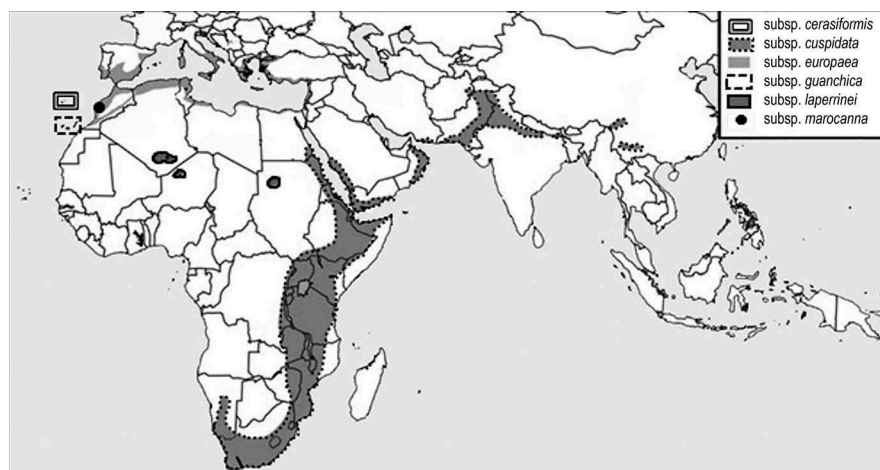


Figure 1. Map of the distribution of *Olea europaea* subspecies. Modified from Rubio de Casas et al. (2006).

The wild olive or oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris* [Miller] Lehr.) and the cultivated olive (*O. europaea* subsp. *europaea* var. *europaea*) (Breton et al. 2006; Belaj et al. 2007) are two co-existing forms of the subspecies *europaea* (Green 2002). Though the exact number is not precisely known, there are at least 1250 *O. europaea* cultivars grown in the Mediterranean countries, and many of them are concentrated in southern Europe: in Italy (538 cultivars), Spain (183), France (88) and Greece (52) (Sarri et al. 2006; Baldoni & Belaj 2009; Ipek et al. 2009).

Over the last two decades, several studies have been carried out in order to investigate how pollen may vary from one cultivar to another (D'Hallewin et al. 1990; Bartolini et al. 1992; Javady & Arzani 2001; Ribeiro et al. 2012). Currently, pollen morphology has been found to be diagnostic at the infrageneric level of some taxa such as family or tribe (Asteraceae, Punt & Hoen 2009; Inuleae, Osman 2006), species (111 species of *Lessingianthus*, Angulo & Dematteis 2010; 33 species of *Justicia*, Rueangsawang et al. 2013; five species of *Muscari*, Ferrauto & Pavone 2016), and cultivars (*Prunus armeniaca* L., Arzani et al. 2005).

Ribeiro et al. (2012) studied pollen morphology, viability and *in vitro* pollen germination of 20 cultivars of olive trees grown in Portugal. Three different groups with similar characteristics (exine reticulum thickness, maximum and minimum diameter) have been identified. Javady and Arzani (2001) characterised five Iranian olive cultivars and concluded that even if individual measurements did not reveal great differences among them, it is possible to delineate a specific cultivar profile by considering all the features together. In a study of 13 Italian cultivars, Roselli (1979) claimed that the identification of some *Olea*

cultivars is not to be considered a difficult task because their pollen features (observed on acetolysed pollen) – including polar axis, equatorial diameter, lumina area and muri thickness – are easily recognisable (cultivars: ‘Nocellara etnea’, ‘Itrana’, ‘Leccino’, ‘Coratina’, ‘Grossa di Spagna’); however, other cultivars look quite indistinguishable (cultivars: ‘Dolce di Bari’, ‘Uovo di Piccione’, ‘Picholine’, ‘Bella di Spagna’, ‘Ascolana’).

In this paper, as further contribution to these studies, the pollen morphology of 14 Italian cultivars of *Olea europaea* has been investigated in order to identify the parameters that may help to discriminate pollen morphological sub-groups within the species. At least one autochthonous cultivar from each main olive-producing region in Italy was selected. All these cultivars are early flowering, well-spread and the majority are dual-purpose cultivars for oil and table olive production. The research has the potentiality to improve the information about morphological profile of olive cultivars grown in the central Mediterranean area. Moreover, the description of main differences in acetolysed pollen from currently cultivated olive trees may help the palaeobotanical or archaeobotanical research in identifying the possible presence of cultivars in the past. This analysis may be a contribution to the reconstruction of the history of cultivation and spread of this tree in the Mediterranean area (Mercuri et al. 2013).

Material and methods

In May 2015, the inflorescences of 14 cultivars were collected from olive trees grown in the experimental fields of the Council for Agricultural Research and Economics – Olive Growing and Oil

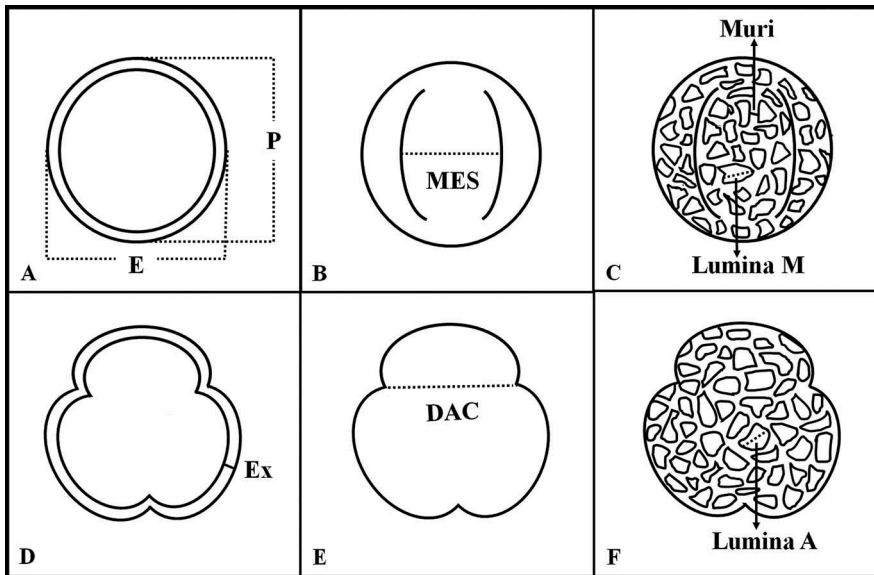


Figure 2. Description of parameters measured using a light microscope. **A–C.** Equatorial view. **D–F.** Polar view. Abbreviations: *E*, equatorial diameter; *P*, polar axis; *MES*, maximum distance between colpi in mesocolpium; *Muri*, exine reticulum thickness in mesocolpium; *Lumina M*, maximum length of lumina in mesocolpium; *Ex*, exine thickness; *DAC*, distance between the apices of two colpi; *Lumina A*, maximum length of lumina in apocolpium.

Industry Research Centre (CREA-OLI), respectively located in Mirto-Crosia and Rende (Cosenza, southern Italy) (Table 1). For each cultivar, three trees were randomly chosen and the flowers were isolated before anthesis with paper bags. The pollen samples were stored at room temperature.

Pollen grains were subjected to dehydration followed by acetolysis (Erdtman 1969). Pollen was mounted in a solution of 1:1 glycerol and water. The observations were taken with a light microscope (LM) at a magnification of 1000×; further examinations were made with a scanning electron microscope (SEM). For LM measurements (Figure 2), ten pollen grains per tree, resulting in 30 pollen grains per cultivar, were measured according to Wrońska-Pilarek et al. (2015). A total of 420 pollen grains were measured. Photographs were taken at a magnification of 1000× with a Leica MC120 HD camera associated with a Leica DMLB light microscope. Measurements were done by using the image analyser software LAS EZ V3.0 by Leica Microsystems. According to Lanza et al. (1996), Javady and Arzani (2001), Al-Dehadheh et al. (2004) and Ribeiro et al. (2012), the morphometric parameters to be considered are polar axis (*P*), equatorial diameter (*E*), *P/E* ratio, maximum distance between colpi in mesocolpium (*MES*), distance between the apices of two colpi (*DAC*), exine thickness (*Ex*), maximum length of lumina in mesocolpium (*Lumina M*) and in apocolpium (*Lumina A*), exine reticu-

lum thickness in mesocolpium (*muri*) and form of mesh.

For SEM observation, acetolysed pollen grains were dehydrated through a graded ethanol series to 100% ethanol, mounted on stubs and coated with a layer of gold using a Emitech K550 sputter coater at the Centre of Inter-Departmental Great Instruments of the University of Modena and Reggio Emilia (CIGS). Pollen grains were observed at 5kV with a Nova Nano SEM 450 (FEI Co., Hillsboro, OR, USA), and were photographed at a magnification of 12 000–13 000× for whole grains, and at 30 000× for exine pattern. Five pollen grains per cultivar were analysed.

The principal component analysis (PCA; PAST, Paleontological Statistics, version 3.11, Hammer et al. 2001) was performed by using the variables together or as separate groups (mean values of *P*, *E*, *P/E*, *MES*, *DAC*, and exine pattern consisting of *Ex*, *Lumina M*, *Lumina A*). The measurements of *muri* were not included because of the risk of preparation artefacts (see later).

Results

All cultivars have trizonocolpate pollen grains except for ‘Carolea’ and ‘Giarraffa’ that have tetrazonocolpate grains (8% of the measured pollen grains; Figure 3). Despite the pollen morphology that looks similar in the different cultivars, the measurements demonstrate that values of the observed parameters are quite variable.

Pollen parameters

The observed ranges are summarised and reported as mean values of the 30 pollen grains per cultivar as obtained from the nine parameters from the 14 cultivars measured using LM (Tables II, III) with the relevant cultivar names reported in brackets: (1) polar axis (*P*) ranges from 21.66 μm ('Tonda di Cagliari') to 25.46 μm ('Pescarese'); (2) equatorial diameter (*E*) ranges from 22.29 μm ('Manna') to 26.61 μm ('Rotondella di Sanza') (Figure 4D, L); (3) the mean values of the *P/E* ratio range from 0.93 to 0.99, this indicates that the pollen grains are oblate spheroidal in shape, according to the terminology of Erdtman (1952); (4) maximum distance between colpi in mesocolpium (MES) ranges from 15.72 μm ('Manna') to 18.38 μm ('Rotondella di Sanza') (Figure 4E, M); (5) distance between the apices of two colpi (DAC) ranges from 10.49 μm ('Manna') to 12.03 μm ('Pescarese'); (6) exine thickness (Ex) varies from quite thick (1.43 μm – 'Manna') to very thick (2.12 μm – 'Rotondella di Sanza'), being of uniform thickness throughout the profile, only in 'Maiatica di Ferrandina', the exine is thicker at the equatorial rather than the polar area (Figure 5); (7) maximum length of lumina in mesocolpium (Lumina M) ranges from 1.76 μm ('Nostrale di Fiano Romano') to 2.24 μm ('Intosso'); (8) maximum length of lumina in apocolpium (Lumina A) varies between 1.60 μm ('Nostrale di Fiano Romano') and 2.14 μm ('Intosso') – the lumina in mesocolpium are always larger than the lumina in apocolpium, in 'Rotondella di Sanza', lumina are similar in apocolpium (1.96 μm) and in mesocolpium (1.97 μm) (Figure 4I, N); (9) exine reticulum thickness in mesocolpium (muri) is variable, ranging from 0.51 μm ('Nostrale di Fiano Romano') to

Table I. Olive cultivars from the CREA-OLI collection, their regions of origin geographically ordered from northern to southern Italy.

Cultivar name	Origin
Santa Caterina (SC)	Tuscany
Nostrale di Fiano Romano (NF)	Lazio
Intosso (I)	Abruzzo
Pescarese (P)	Abruzzo
Rotondella di Sanza (RS)	Campania
Carolea (C)	Calabria
Grossa di Cassano (GC)	Calabria
Maiatica di Ferrandina (MF)	Basilicata
Giarrappa (G)	Sicily
Nocellara messinese (NM)	Sicily
Nocellara del Belice (NB)	Sicily
Santagatese (SA)	Sicily
Manna (M)	Sardinia
Tonda di Cagliari (TC)	Sardinia

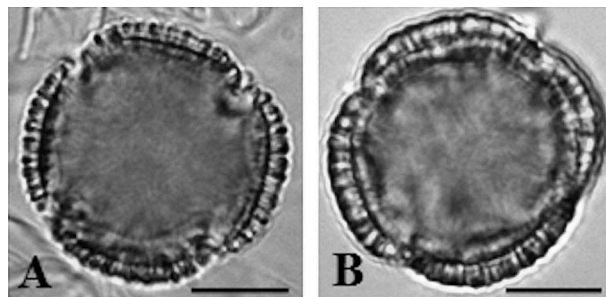


Figure 3. Light micrographs of tetrazonocolpate pollen grains in polar view. **A.** Cultivar 'Carolea'. **B.** Cultivar 'Giarrappa'. Scale bars – 10 μm .

0.64 μm ('Rotondella di Sanza' and 'Nocellara messinese'). Pollen grains show low variability in size (*P/E* ratio: 0.93–0.99) and homogeneity in shape (oblate spheroidal) (Table II).

Exine pattern

The pattern of exine consisted in a continuous, fine or coarse reticulum with more or less sculptured muri delimiting lumina area, which had various forms and dimensions in the different cultivars. The pattern shows several differences in the exine and muri thickness, maximum length of lumina in mesocolpium and in apocolpium, and in the form of meshes. In fact, meshes are irregular and two main types were recognised: the isodiametric type, as in 'Carolea' and 'Giarrappa', and the elongated type, as in 'Pescarese' and 'Intosso' (Table III).

Two very similar profiles of exine pattern have been revealed through LM in 'Grossa di Cassano' and 'Nocellara del Belice' that have similar dimensions (Figure 6). From SEM observations, all cultivars present transversal ridges on the muri (Figures 7, 8B, D); both of the cultivars at the *E* extremes, from the maximum ('Rotondella di Sanza') to minimum equatorial diameter ('Manna'), show muri that are similar in width but different in height (in 'Rotondella di Sanza' muri are higher than in 'Manna') (Figure 7B, D).

Statistical analysis

The PCA of all parameters (*P*, *E*, *P/E*, MES, DAC, Ex, Lumina M, Lumina A; Figure 9A) shows that the two principal components are responsible for 87.9% and 6.5% of variation. The cultivars are preferably distributed along component 2, but variables involved in this distribution are not clearly appreciable. The PCA of *P*, *E*, *P/E*, MES and DAC only (Figure 9B) shows

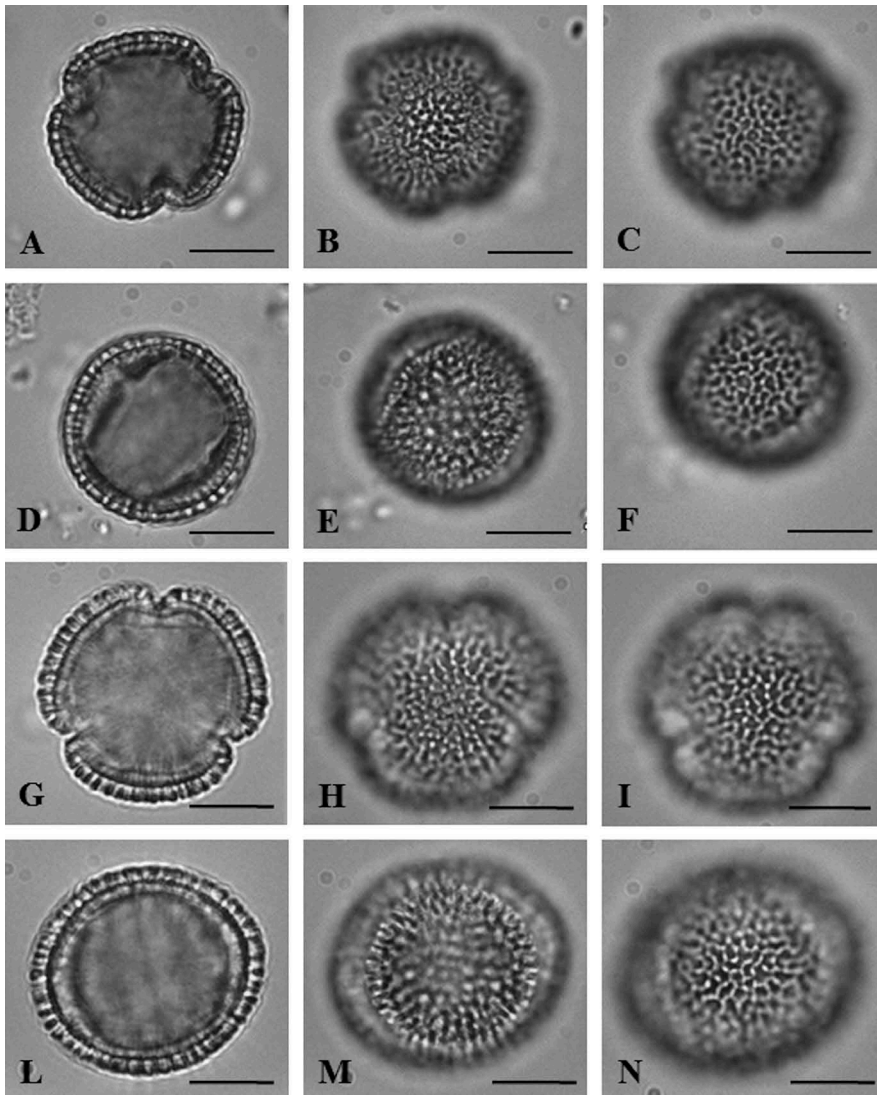


Figure 4. Light micrographs of cultivars 'Manna' and 'Rotondella di Sanza'. **A–C.** Polar view of cultivar 'Manna'. **D–F.** Equatorial view of cultivar 'Manna'. **G–I.** Polar view of cultivar 'Rotondella di Sanza'. **L–N.** Equatorial view of cultivar 'Rotondella di Sanza'. Scale bars – 10 μ m.

that the two axes are responsible for 89.6% and 6.3% of the total variation, respectively. The ordination of the cultivars along axis 2 reflects a grain size-based gradient, from the smallest pollen (left) to the largest one (right). The distribution along axis 1 does not correspond to one specific parameter and seems to be linked to a pattern of characters that are not clearly identifiable. Overall, the PCA distribution shows that three different pollen groups can be identified (circles in [Figure 9B](#)).

In the PCA processing of exine parameters (Ex, Lumina M, Lumina A; muri excluded), the two axes are responsible for 77.7% and 20.2% of the total variation, respectively ([Figure 9C](#)). In the four sectors, distribution is independent from a single character;

consequently, it is not possible to identify a specific connection between the parameters and PCA axes. The exine pattern PCA allows recognises two different types of pollen grains on the basis of the maximum length of lumina M, lumina A and the exine thickness (circles in [Figure 9C](#)).

Discussion

The pollen grains of the 14 studied cultivars seem quite similar but main morphological differences can be recognised in the size and exine pattern at microscope observation. Moreover, the statistical elaboration point to the discriminative potential of polar axis and equatorial diameter. Considering each pollen parameter, measures are close between each

other. They look ineffective in discriminating types among the cultivars. For example, *P* (polar axis) of both ‘Nocellara del Belice’ and ‘Giarraffa’ measures 23.87 μm on average. The *E* (equatorial diameter) of ‘Intosso’ measures 24.77 μm , a value that is very similar to the 24.73 μm of ‘Nocellara del Belice’. The maximum distance between colpi in mesocolpium of ‘Pescarese’ and ‘Nostrale di Fiano Romano’ is 17.64 μm , the same in both cultivars (Table II). The measure of exine reticulum thickness in mesocolpium is the same in ‘Santa Caterina’ and in ‘Grossa di Cassano’ (0.55 μm). ‘Manna’ and ‘Santagatese’ have similar exine thickness (1.43 μm and 1.49 μm , respectively) and maximum length of lumina in apocolpium (1.76 μm and 1.74 μm) (Table III).

According to Erdtman (1945), *Olea* has a small pollen ($\leq 25 \mu\text{m}$), with few exceptions belonging to the medium size (c. 26 μm). Nevertheless, according to the mean values of polar axis and equatorial diameter, three different pollen groups can be identified: small (*P*: 21.75 μm , *E*: 22.55 μm), cultivars ‘Manna’ and ‘Tonda di Cagliari’; large (*P*: 25.1 μm , *E*: 26.1 μm), cultivars ‘Pescarese’ and ‘Rotondella di Sanza’; medium (*P*: 23.49 μm , *E*: 24.57 μm), cultivars ‘Grossa di Cassano’, ‘Nocellara del Belice’, ‘Santagatese’, ‘Nostrale di Fiano Romano’, ‘Carolea’, ‘Giarraffa’, ‘Nocellara messinese’, ‘Intosso’, ‘Maiatica di Ferrandina’, ‘Santa Caterina’ (Table II).

The exine pattern allows recognising different types of pollen grains on the basis of the maximum length of lumina (M and A, both ranging from 1.60 to 2.24 μm) and the exine thickness: there are four cultivars with small lumina M (1.76–1.88 μm) and lumina A (1.60–1.75 μm): ‘Grossa

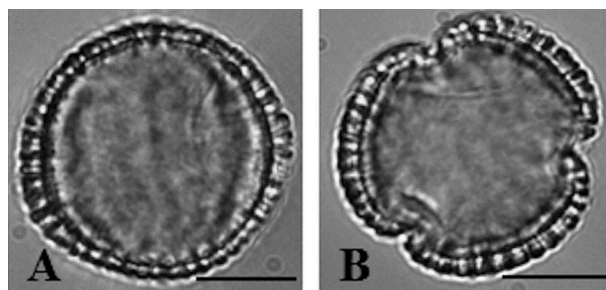


Figure 5. Light micrographs of cultivar ‘Maiatica di Ferrandina’. **A.** Equatorial view. **B.** Polar view. Scale bars – 10 μm .

di Cassano’, ‘Nocellara del Belice’, ‘Santagatese’, ‘Nostrale di Fiano Romano’; in this group, ‘Grossa di Cassano’, ‘Nocellara del Belice’ and ‘Santagatese’ have thin exine (1.49–1.54 μm) while ‘Nostrale di Fiano Romano’ has a relatively medium thickness exine (1.69 μm); ten cultivars have large lumina M (1.92–2.24 μm) and lumina A (1.76–2.14 μm): ‘Carolea’, ‘Giarraffa’, ‘Nocellara messinese’, ‘Manna’, ‘Tonda di Cagliari’, ‘Intosso’, ‘Pescarese’, ‘Maiatica di Ferrandina’, ‘Santa Caterina’, ‘Rotondella di Sanza’; in this group, two cultivars have a relatively thin exine (1.43–1.58 μm ; ‘Manna’, ‘Tonda di Cagliari’), four have a medium-thickness exine (1.66–1.83 μm ; ‘Carolea’, ‘Intosso’, ‘Nocellara messinese’, ‘Pescarese’) and the other four have a thick exine (1.98–2.12 μm), ‘Giarraffa’, ‘Maiatica di Ferrandina’, ‘Santa Caterina’, ‘Rotondella di Sanza’ (Table III).

By combining several parameters, each cultivar shows a specific profile with a singular pattern of parameters (Tables II, III). The pollen of ‘Roton-

Table II. Morphometric data of pollen grains from olive cultivars (measurements are taken with light microscopy).

Cultivar	<i>P</i> (μm)	<i>E</i> (μm)	<i>P/E</i>	Shape	MES (μm)	DAC (μm)
Santa Caterina	23.94 $\pm$ 1.31	25.64 $\pm$ 1.36	0.93	Oblate spheroidal	18.36 $\pm$ 1.76	11.65 $\pm$ 0.99
Nostrale di Fiano Rom.	22.99 $\pm$ 1.43	24.06 $\pm$ 1.31	0.95	Oblate spheroidal	17.64 $\pm$ 1.01	11.16 $\pm$ 0.74
Intosso	23.47 $\pm$ 1.15	24.77 $\pm$ 1.25	0.95	Oblate spheroidal	17.45 $\pm$ 1.04	10.82 $\pm$ 0.78
Pescarese	25.46 $\pm$ 1.90	25.59 $\pm$ 1.51	0.99	Oblate spheroidal	17.64 $\pm$ 1.32	12.03 $\pm$ 1.35
Rotondella di Sanza	24.74 $\pm$ 1.69	26.61 $\pm$ 1.16	0.93	Oblate spheroidal	18.38 $\pm$ 1.62	11.84 $\pm$ 0.77
Carolea	22.09 $\pm$ 0.99	23.60 $\pm$ 1.51	0.94	Oblate spheroidal	16.22 $\pm$ 1.46	11.20 $\pm$ 1.24
Grossa di Cassano	23.61 $\pm$ 1.40	24.40 $\pm$ 1.36	0.97	Oblate spheroidal	17.73 $\pm$ 0.92	11.80 $\pm$ 0.74
Maiatica di Ferrandina	24.06 $\pm$ 1.56	25.52 $\pm$ 1.91	0.95	Oblate spheroidal	17.93 $\pm$ 1.99	11.51 $\pm$ 1.28
Giarraffa	23.87 $\pm$ 1.50	25.21 $\pm$ 1.51	0.95	Oblate spheroidal	18.22 $\pm$ 0.97	11.06 $\pm$ 1.15
Nocellara messinese	23.64 $\pm$ 1.35	24.28 $\pm$ 1.55	0.97	Oblate spheroidal	17.50 $\pm$ 1.36	11.63 $\pm$ 0.96
Nocellara del Belice	23.87 $\pm$ 0.91	24.73 $\pm$ 1.23	0.97	Oblate spheroidal	17.98 $\pm$ 0.91	11.36 $\pm$ 0.84
Santagatese	23.35 $\pm$ 1.21	23.48 $\pm$ 1.02	0.99	Oblate spheroidal	16.73 $\pm$ 1.02	11.56 $\pm$ 0.73
Manna	21.85 $\pm$ 1.65	22.29 $\pm$ 1.93	0.98	Oblate spheroidal	15.72 $\pm$ 1.48	10.49 $\pm$ 0.96
Tonda di Cagliari	21.66 $\pm$ 1.46	22.82 $\pm$ 1.33	0.95	Oblate spheroidal	16.41 $\pm$ 1.36	10.65 $\pm$ 1.80

Note: Each value is the average of 30 individual measurements $\pm$ standard deviation. *P*, polar axis; *E*, equatorial diameter; MES, maximum distance between colpi in mesocolpium; DAC, distance between the apices of two colpi.

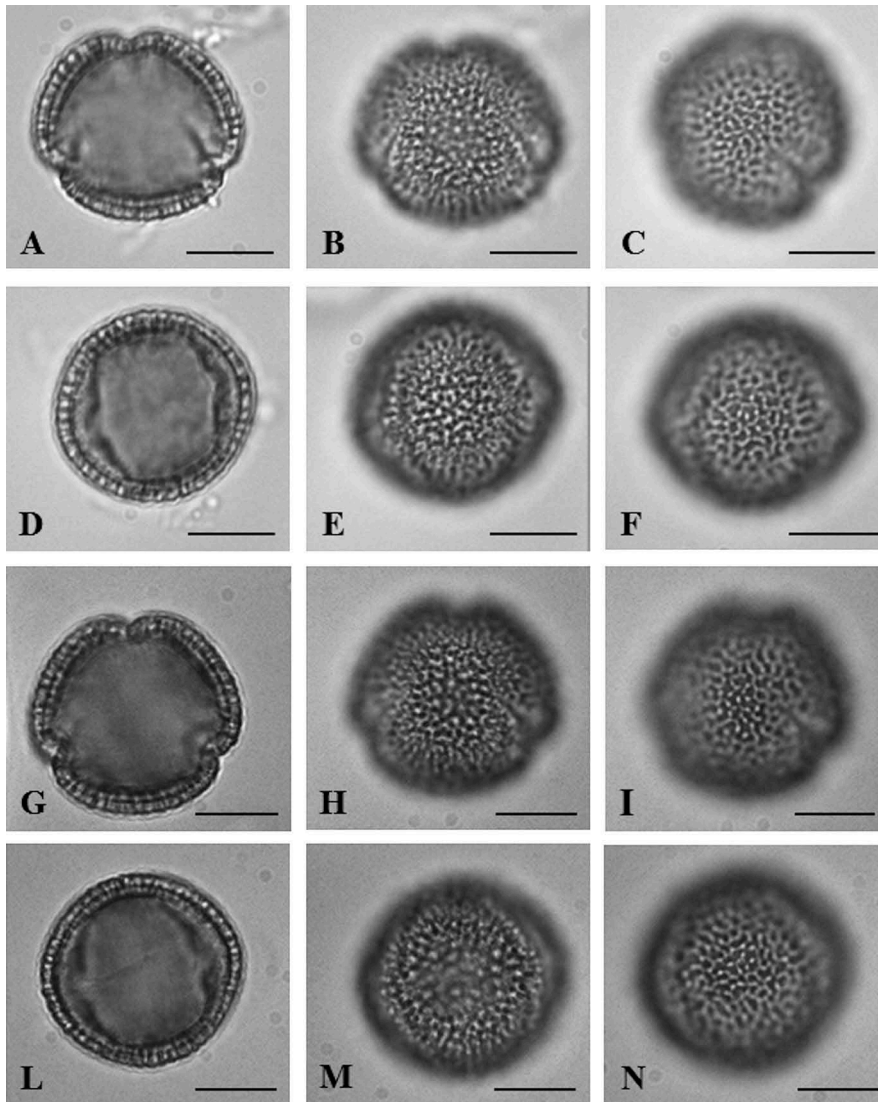


Figure 6. Light micrographs of cultivars 'Grossa di Cassano' and 'Nocellara del Belice'. **A–C.** Polar view of cultivar 'Grossa di Cassano'. **D–F.** Equatorial view of cultivar 'Grossa di Cassano'. **G–I.** Polar view of cultivar 'Nocellara del Belice'. **L–N.** Equatorial view of cultivar 'Nocellara del Belice'. Scale bars – 10 μm .

della di Sanza' has large pollen (P : 24.74 μm , E : 26.61 μm) with the thickest exine (2.12 μm), and also the other parameters have very high values, except for the maximum length of lumina. In particular, its muri look thicker than those observed in other cultivars, such as 'Manna' using LM, while appear similar after SEM observation (Figure 7). It could be argued that the muri of 'Rotondella di Sanza' are high (about 1.80 μm) and when pollen grains are observed by means of LM, the coverslip may press them creating an artefact. For this reason, due to the conflicting results coming from SEM and LM analysis, muri thickness measured by LM was not considered reliable in discriminating *Olea europaea* cultivars, and was excluded from the PCA.

On the contrary, 'Manna' has among the smallest pollen grains (P : 21.85 μm , E : 22.29 μm), showing low values for all parameters except for the lumina length in mesocolpium and apocolpium; the latter parameters (lumina M: 1.92 μm , lumina A: 1.76 μm) are similar to the mean values of the other cultivars. 'Santa Caterina' has medium size (P : 23.94 μm , E : 25.64 μm), large lumina in mesocolpium and apocolpium, large MES but thin muri. 'Giarraffa' is similar although MES and DAC are smaller than those observed in the pollen of 'Santa Caterina'.

The PCA based on pollen features (Figure 9B) suggests that P and E are efficient parameters to discriminate between cultivars. This is in contrast with Lanza et al. (1996) who did not report on significant differences with regard to

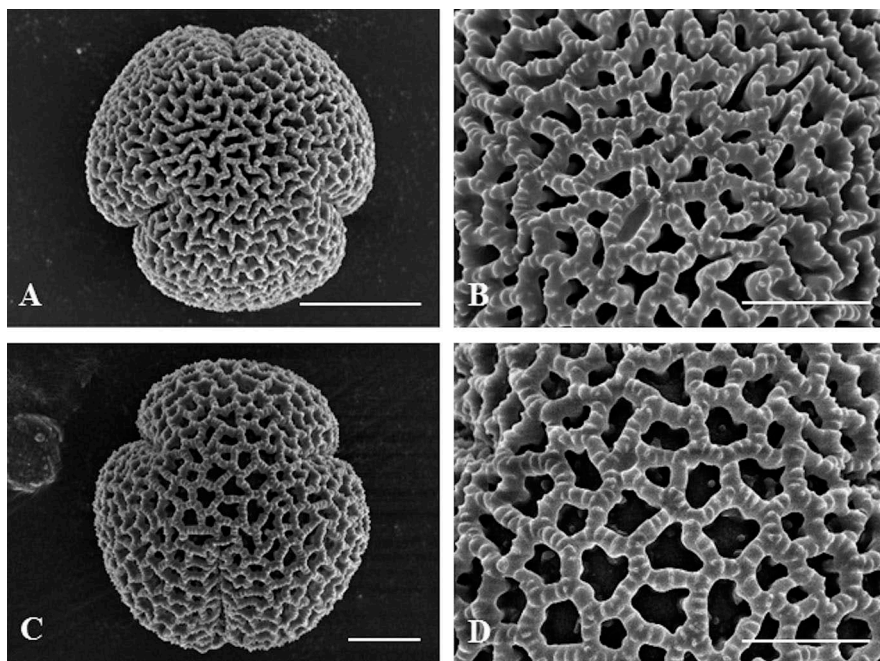


Figure 7. Scanning electron micrographs of cultivars 'Rotondella di Sanza' and 'Manna'. **A.** Whole grain of cultivar 'Rotondella di Sanza'. **B.** Exine surface of cultivar 'Rotondella di Sanza'. **C.** Whole grain of cultivar 'Manna'. **D.** Exine surface of cultivar 'Manna'. Scale bars – 10 µm (A), 5 µm (C), 4 µm (B, D).

polar and equatorial diameters by investigating four *Olea* cultivars ('Grossa di Cassano', 'Picholine', 'Leccino', 'Coratina'). In this study, the PCA axis 2 separates the cultivars along a grain size-based gradient: pollen characterised by the highest values of *P* and *E* are grouped in the first and fourth sectors of the PCA. These observations are consistent with the results of the studies by Roselli (1979) and Koubouris et al. (2012).

Our statistical analysis confirms that each cultivar exhibits a singular exine pattern. Actually, this feature is considered a good discriminant parameter to distinguish pollen types among olive cultivars (Pacini & Vosa 1979; Lanza et al. 1996; Koubouris et al. 2012) as well as in other plant species (apple clones: Marcucci et al. 1984; basmati rice: Datta & Chaturvedi 2004). The PCA distribution shows that some olive cultivars, such as 'Manna' and 'Rotondella di Sanza', have very different profiles of combined parameters, while others such as 'Grossa di Cassano' and 'Nocellara Messinese' are fairly indistinguishable. Moreover, the PCA based on exine characters (Figure 9C) suggests that exine thickness and maximum length of lumina in mesocolpium and apocolpium are independent characters. The distribution of cultivars in the four sectors of this PCA indicates that a predominant character that discriminate between cultivars does not exist.

Conclusions

Within basic pollen morphology of small and reticulate pollen, dimensions, exine thickness and maximum length of lumina are the principal parameters, which can be used for discriminating cultivars from olive pollen grains. The easiest way to classify pollen from different cultivars is to measure *P* and *E* length observed using LM. Based on the maximum length, our study allows to identify three groups of small (< 23 µm; 'Manna' and 'Tonda di Cagliari'), medium (23–25 µm; 'Carolea', 'Grossa di Cassano', 'Giarraffa', 'Nocellara messinese', 'Nocellara del Belice', 'Santagatese', 'Intosso', 'Maiatica di Ferlandina', 'Nostrale di Fiano Romano', 'Santa Caterina') and large (> 25 µm; 'Pescarese' and 'Rotondella di Sanza') pollen grains. Three further sub-groups may be identified within the medium group, based on the length of lumina and exine thickness: (1) medium cultivars with small lumina M (1.76–1.88 µm), small lumina A (1.60–1.75 µm) and fine exine thickness (1.49–1.54 µm): 'Grossa di Cassano', 'Nocellara del Belice' and 'Santagatese'; 'Nostrale di Fiano Romano' has a medium-thickness exine (1.69 µm); (2) medium cultivars with large lumina M (1.94–2.24 µm), large lumina A (1.83–2.14 µm) and medium-thickness exine (1.66–1.77 µm): 'Carolea', 'Intosso', 'Nocellara messinese'; (3) medium cultivars with large

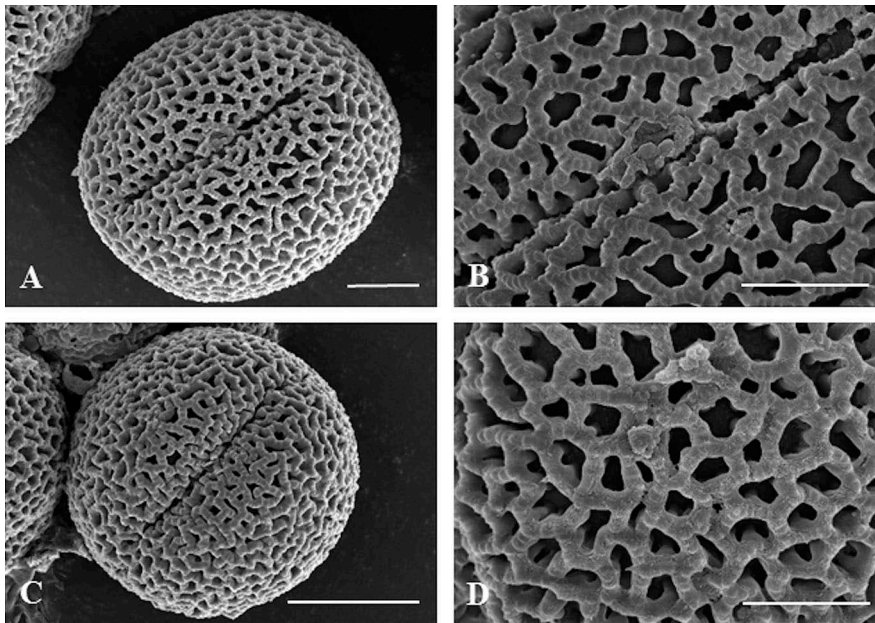


Figure 8. Scanning electron micrographs of cultivars 'Grossa di Cassano' and 'Nocellara del Belice'. **A.** Whole grain of cultivar 'Grossa di Cassano'. **B.** Exine surface of cultivar 'Grossa di Cassano'. **C.** Whole grain of cultivar 'Nocellara del Belice'. **D.** Exine surface of cultivar 'Nocellara del Belice'. Scale bars – 10 µm (C, D), 5 µm (A), 4 µm (B).

lumina M (2.13–2.20 µm), large lumina A (1.89–2.13 µm) and thick exine (1.98–2.08 µm): 'Giarraffa', 'Maiatica di Ferrandina', 'Santa Caterina' (Table III). Moreover, in the exine pattern, two main types of irregular meshes were recognised: the isodiametric type, as in 'Carolea' and 'Giarraffa', and the elongated type, as in 'Pescarese' and 'Intosso'. These results are consistent with Roselli (1979) and Ribeiro et al. (2012) who combine dimension and exine reticulum thickness to subdivide pollen of *Olea* cultivars into morphological groups. A single

character often presents quite similar values in different cultivars, but the overall consideration of the morphological parameters shows that each cultivar has a well-defined pattern of parameters (Tables II, III). Our results support the conclusions by Javady and Arzani (2001): measurements made on olive pollen grains (*P*, *E*, *P/E* ratio and exine pattern) are not individually discriminant for cultivars; when all features are considered together it is possible to delineate a specific pollen profile for each cultivar that can be applied to cultivar identification.

Table III. Exine patterns of pollen grains from olive cultivars (measurements are taken with light microscopy).

Cultivar	Ex (µm)	Muri (µm)	Lumina M (µm)	Lumina A (µm)	Form of mesh
Santa Caterina	2.08 ± 0.12	0.55 ± 0.05	2.20 ± 0.42	2.13 ± 0.39	Isodiametric
Nostrale di Fiano Rom.	1.69 ± 0.12	0.51 ± 0.05	1.76 ± 0.33	1.60 ± 0.27	Isodiametric
Intosso	1.77 ± 0.08	0.60 ± 0.06	2.24 ± 0.38	2.14 ± 0.43	Elongated
Pescarese	1.83 ± 0.13	0.59 ± 0.07	2.05 ± 0.43	1.90 ± 0.42	Elongated
Rotondella di Sanza	2.12 ± 0.19	0.64 ± 0.06	1.97 ± 0.38	1.96 ± 0.35	Isodiametric
Carolea	1.74 ± 0.12	0.56 ± 0.07	1.94 ± 0.39	1.83 ± 0.41	Isodiametric
Grossa di Cassano	1.52 ± 0.11	0.55 ± 0.05	1.88 ± 0.35	1.75 ± 0.38	Elongated
Maiatica di Ferrandina	1.98 ± 0.16	0.59 ± 0.07	2.13 ± 0.38	1.89 ± 0.37	Isodiametric
Giarraffa	2.02 ± 0.18	0.61 ± 0.08	2.16 ± 0.41	2.06 ± 0.39	Isodiametric
Nocellara messinese	1.66 ± 0.11	0.64 ± 0.06	2.21 ± 0.44	1.96 ± 0.37	Elongated
Nocellara del Belice	1.54 ± 0.18	0.53 ± 0.05	1.85 ± 0.35	1.72 ± 0.33	Elongated
Santagatese	1.49 ± 0.16	0.57 ± 0.06	1.85 ± 0.40	1.74 ± 0.34	Isodiametric
Manna	1.43 ± 0.11	0.53 ± 0.05	1.92 ± 0.38	1.76 ± 0.38	Isodiametric
Tonda di Cagliari	1.58 ± 0.11	0.61 ± 0.05	2.18 ± 0.40	1.88 ± 0.36	Elongated

Note: Each value is the average of 30 individual measurements ± standard deviation. Ex, exine thickness; Muri, exine reticulum thickness in mesocolpium; Lumina M, maximum length of lumina in mesocolpium; Lumina A, maximum length of lumina in apocolpium.

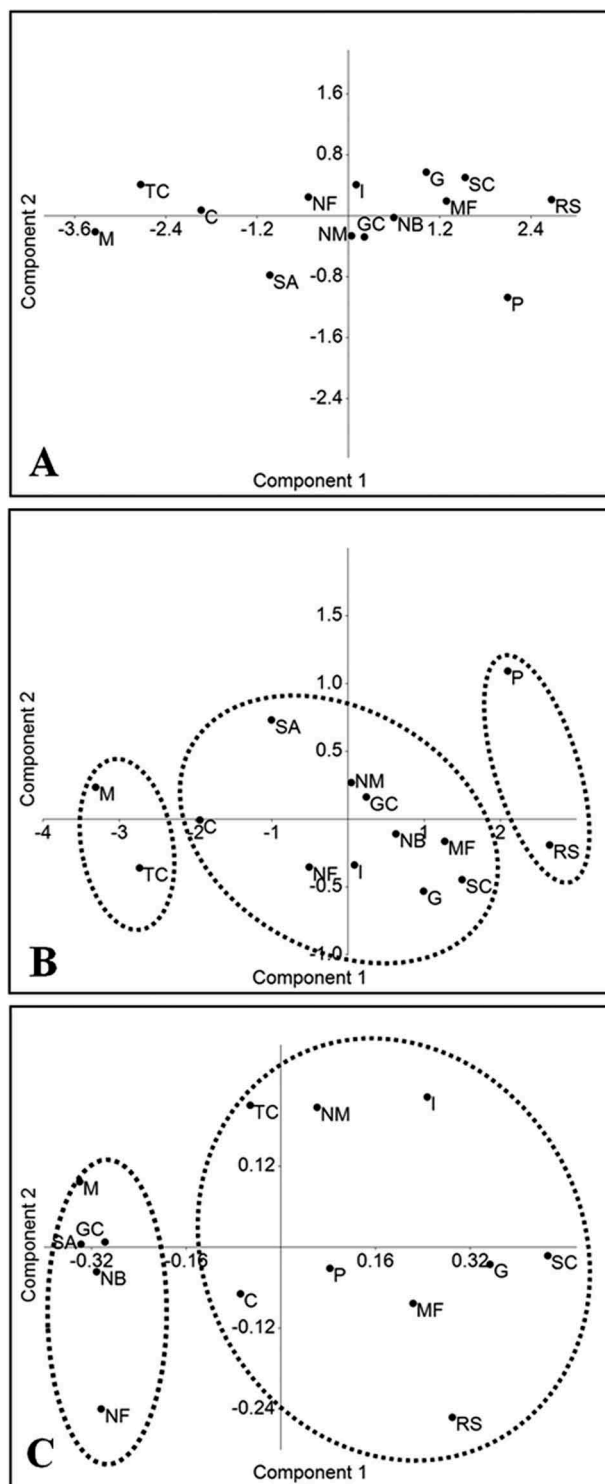


Figure 9. Principal component analysis. **A.** Principal component analysis of pollen grain size parameters (*P*, polar axis; *E*, equatorial diameter; *P/E*, maximum distance between colpi in mesocolpium; distance between the apices of two colpi) and of exine parameters (exine thickness; maximum length of lumina in mesocolpium; maximum length of lumina in apocolpium). **B.** Principal component analysis of pollen grain size parameters as in (A). **C.** Principal component analysis of the exine parameters as in (A). Names of cultivars are labelled following codes in Table I.

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Supplemental data

Supplemental data for this article can be accessed [here](#).

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