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Pollen Morphology of Five Iranian Olive *(Olea europaea* L.*)* Cultivars

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ABSTRACT

Pollen grains of five olive cultivars *(Olea europaea* L .*)* were examined, using Scanning Electron Microscopy (SEM) for identification purposes. Four measurments, polar axis (P), equatorial diameter (E), P/E ratio and exine patterns were made. Forty pollen grains of each cultivar were viewed before finally selecting a representative pollen grain. Polar diameter ranged between 22.76 μm for the 'Mary' and 28.58 μm for the 'Shengeh' 'Zard' and 'Roghani' cultivars exhibited incomplete closure of the meshes. 'Zard' and 'Shengeh' cultivars had thick muri but 'Roghani', 'Mary' and 'Fishomi' possessed thinner muri. Scanning electron analysis of pollen grain revealed that specific differences including variation in size and form of the meshes are exist among cultivars. The differences in the exine pattern may help for the identification of some cultivars.

Keywords: Exine pattern, Olive *(Olea europaea* L. *),* Pollen analysis, Pollen grain, Scanning Electron Microscopy (SEM).

INTRODUCTION

Taxonomists and paleobotanists have recognized the importance of pollen development and morphology in clarifying the classification of plants (Martens and Fretz, 1980). Scanning electron microscopy studies on cultivated fruit trees have been carried out for taxonomic purposes using pollen (Lanza *et al.,* 1996). The studies were undertaken to determine interspecific and intraspecific relationships between fruit tree species. A number of specific pollen characteristics have been cited as useful for differentiation between closely related plant groups. Several workers suggested that pollen morphology, including size, shape and exine striation patterns may be useful for species identification (Martens and Fretz, 1980, Maas, 1977).

Pacini and Vosa (1979) studied exine pattern in nine olive cultivars using SEM. They observed that differences in exine pattern among various cultivars mirror differences in size and shape of leaves and fruit, as well as in the general appearance of the tree. Westwood and Challice (1978) characterized pyrus species by observing pollen grain size and shape in addition to exine features such as ridge prominence, pore, as well as pit presence. Fogle (1977a, b) compared pollen of several fruit tree species, including peach, nectarine, plum, apple and sweet cherry through exine details obtained by scanning electron microscopy.

Differences in exine patterns between some cultivars have been noted in some species (Fogle, 1977, Maas, 1977) but not all cultivars exhibit very differentiated patterns. Recently, the isozyme techniques and random amplified polymorphic DNA (RAPD) markers have been used to distinguish fruit cultivars (Bogani, *et al*., 1994, Ouazzani, *et al.,* 1994, Patumi, *et al*., 1994; Wiesman *et*

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Table 1. Characteristics of five olive cultivar pollen grains.

Cultivars	Pollen size (μ m) Polar axis (P) [a]	Equatorial diam (E)[a]	Distance between furrow edges[a]	P/E	Shape
Shengeh	25.58 ± 0.87a[b]	19.14 ± 0.93a	13.81 ± 1.01a	1.339b	prolate
Zard	25.16 ± 1.34a	17.48 ± 1.44ab	11.707 ± 1.05b	1.447ab	prolate
Roghani	23.77 ± 0.82ab	16.69 ± 1.02b	11.55 ± 0.84b	1.429ab	prolate
Fishomi	23.46 ± 1.18ab	15.95 ± 0.91b	10.994 ± 0.91b	1.473a	prolate
Mary	22.76 ± 3.01b	16.85 ± 2.51b	10.965 ± 1.88b	1.357b	prolate

[a]Each value represents the mean of 10 individual measurements ± SD
[b] Mean separation in columns by Duncan's Multiple Range Test at (P=0.01)

al., 1998). For taxonomic purposes the best results are achieved by combining evidence from as many different fields or levels of organization as possible, such as morphological evidence, isozyme analysis and RAPD.

Olive is one of the fruit trees which will become important in the Iranian fruit industry in a near future. As a preliminary study, pollen exine structure was examined in five Iranian native olive cultivars using SEM technique.

MATERIALS AND METHODS

Pollen was collected in 1998 from olive trees (*Olea europaea* L.), cvs. Zard, Mary, Roghani, Fishomi and Shengeh grown in the orchard of Roodbar Olive Research Station located in Roodbar, Gailan province, Iran. Three branches selected from various parts of each tree (three to each cultivar) were isolated, before anthesis, with paper bags to avoid contamination from other pollen sources. The anthers were kept dry to allow pollen release. Pollen was collected in small vials and stored in desiccator at 3-5 °C until used.

For SEM evaluation, pollen samples were shaken for 30 minutes in a mixture of 1:1 chloroform and carbon disulphide in order to remove the triphyne as described by Pacini and Juniper method (1979).

Pollen grains were mounted on aluminium stubs with double-faced transparent tape and were coated with a gold layer of 200°A thick in a sputter coater. Samples on SEM stubs were stored in a desiccator perior to and following coating. Pollen grains observed at 20 KV with a Stereoscan 360 SEM were photographed at 5000× for whole grain and 15000× for exine pattern. Forty pollen grains of each cultivar were viewed before selecting as a representative pollen grain. Ten samples were measured in each cultivar. The polar axis, equatorial diameter and distance between two furrows were measured. The data were analysed and examined by analysis of variance and the means compared, using Duncan's Multiple Range Test.

RESULTS

There are 4 morphological features important to the description of olive pollen, 1) shape, 2) sculpturing of the exine, 3) size, and finally 4) P/E ratio. The differences between means, comparison of meseared char-

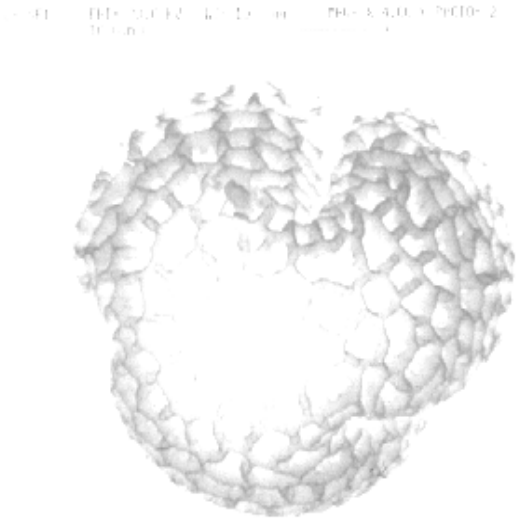

Figure 1. Polar veiw of olive pollen grain cv. Zard

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Figure 2. Pollen of olive, cv. Zard. Magnification: (a)× 4000; (b)× 15000. Scale represents 10 μm in photomicrograph (a) and 2μm in (b).

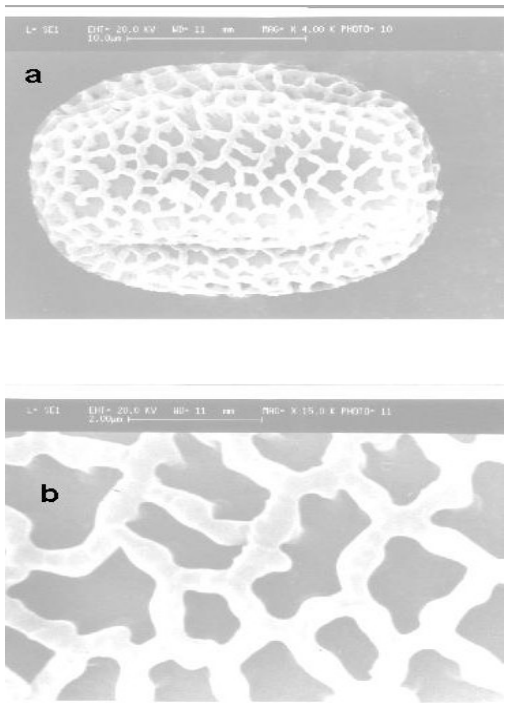

Figure 3. Pollen of olive, cv. 'Roghani'. Magnification: (a)× 4000; (b)× 15000. scale represents 10 μm in photomicrograph (a) and 2 μm in (b).

acters, are shown in Table 1. The pollens of investigated cultivar showed diversity in shape, size, P/E ratio and exine characteristics (Table 1). Pollen grains in all cultivars were trizonocolpate (Fig.1). The outstanding characteristic of these grains was the reticulate exine.

The mean values of polar diameter ranged between 22.76 μm for 'Mary' and 28.58 μm for 'Shengeh' while equatorial diameter ranging between 15.95 μm for ''Fishomi' and 19.14 μm for 'Shengeh'. According to Erdtman (1971) terminology, pollen grains were placed in 1 shape group based on the P/E ratio (Table 1). The P/E ratio ranged from 1.34 to 1.47.

Results indicated that differences in exine pattern in studied cultivars were included three features, these being incomplete meshes, thickness of muri and mesh density in unit area. The incomplete closure of the meshes are found in 'Zard' and 'Roghani' cultivars (Fig. 2 a,b; Fig. 3 a,b). The cultivars with thicker muri are 'Zard' and 'Shengeh' (Fig. 2a,b; Fig. 4 a,b). Cultivars with thinner muri are 'Roghani', 'Mary' and 'Fishomi' (Fig. 3 a,b; Fig. 5 a,b; Fig. 6 a,b).

The other important character was mesh density in which pollen of all the studied cultivars were placed in the following two groups:

1) Low density of mesh in unit area: Cvs. 'Mary', and 'Shengeh' belonged to this group (Fig. 5a,b; Fig. 4a,b).

2) High density of mesh in unit area: Cvs. 'Zard', `Roghani', and 'Fishomi' belonged to this group (Fig. 2a,b; Fig. 3a,b; Fig. 6a,b).

DISCUSSION

This study supports the conclusion in Bohra and Sharma (1979) studies who reported that differences in exine patterns of olive cultivars including differences in dimension and shape of mesh, thickness of muri can be useful for the determination of

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Figure 4. Pollen of olive, cv. 'Shengeh'. Magnification: (a)× 4000; (b)× 15000. Scale represents 10 μm in photomicrograph (a) and 2μm in (b).

olive cultivars.

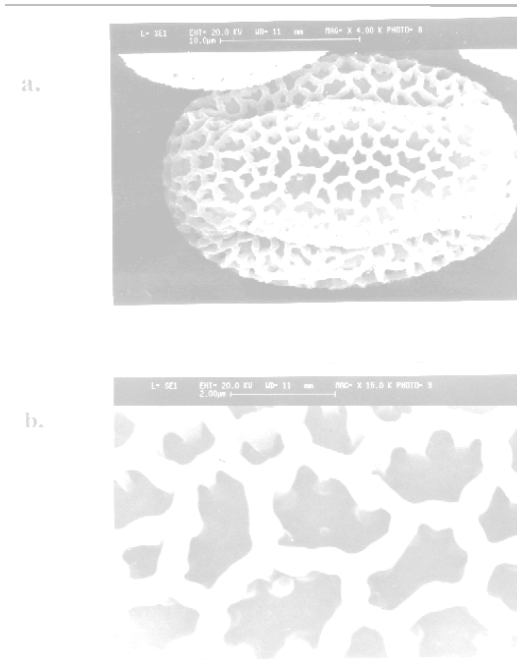

Figure 5. Pollen of olive, cv. 'Mary'. Magnification: (a)× 4000; (b)× 15000. Scale represents 10 μm in photomicrograph (a) and 2μm in (b).

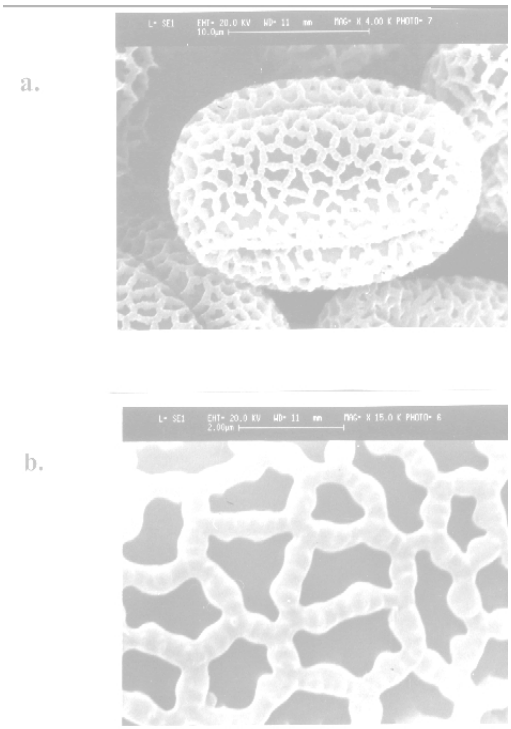

Figure 6. Pollen of olive, cv. Fishomi. Magnification: (a)× 4000; (b)× 15000. Scale represents 10 μm in photomicrograph (a) and 2μm in (b).

Large meshes were present in `Shengeh' and 'Mary' while the muri were thick in `Shengeh'cultivar, this character being constant in the former cultivars. Bohra and Sharma (1979) reported thicker muri in `S. Francesco'and `S. Agostino' while thinner muri in 'Grappolo' and `Cornila' olive cultivars. P/E ratio based on means of pollar axis and equatorial diameter ranged from 1.34 to 1.47. The range for P/E ratio for pollen in 4 olive cultivars reported by Lanza *et al.* (1995) was from 1.32 for the `Picoline' to 1.37 for the `Leccino'.

A useful trait, from the ultrastructural point of view was mesh density in unit area. This trait permits the separation of 'Mary' and 'Shengeh' from 'Zard', 'Roghani and `Fishomi' cultivars. Differences in exine pattern among some cultivars have been noted in olive, grapes, and in apple clones (Bohra and Sharma, 1979; Ahmedullah, 1983; Marcucci *et al*., 1984).

Four measurements made on pollen grains (polar axis, equatorial diameter, P/E ratio

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and exin patterns) did not individually reveal great differences among cultivars. However, when all features are considered together the pollen of each cultivar exhibits a unique pattern. Thus, these pollen features along with morphological characteristics of tree can be employed for cultivar identification.

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مورفولوژی دانه گرده پنج رقم زیتون (*Olea europaea* L.) ایران

چکیده

به منظور شناسایی مرفولوژی دانه‌های گرده، نمونه‌هایی از دانه گرده پنج رقم زیتون (*Olea europaea* L.)، با استفاده از میکروسکوپ الکترونی (SEM) بررسی شدند. اندازه گیری بر روی محور قطبی (P)،

قطر استوایی (E)، نسبت P/E و الگوی اگزین دانه‌های گرده انجام گرفت. قبل از انتخاب دانه گرده نمونه برای اندازه‌گیریهای نهایی، حداقل ٤٠ دانه گرده از هر رقم بازبینی شدند. طول محور قطبی معادل ٢٢/٧٦ µm در رقم ماری ٢٨/٥٨ µm در رقم شنگه بود. بر روی دانه گرده ارقام زرد و روغنی شبکه های ناکامل وجود داشت. بر روی دانه گرده ارقام زرد وشنگه خطوط برجسته ضخیم مشاهده گردید. اما در ارقام روغنی، ماری و فیشمی خطوط برجسته روی گرده نازکتر بود. آنالیز میکروسکوپی دانه گرده تفاوتهای خاصی را شامل گوناگونی در اندازه و شکل شبکه‌های روی دانه گرده نشان داد. نتایج حاصله نشان داد که از الگوی اگزینی دانه گرده ارقام مورد مطالعه می‌توان در مطالعات شناسایی ارقام کمک گرفت.

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