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Investigations on the Pollen Morphology of Some Fruit Species

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Abstract: Morphological characters are used to identify plant material. Pollen morphology is an important parameter. This is of great importance in detailed investigations by scanning electron microscope (SEM). In order to develop a plant material identification tool, this research was carried out to determine the pollen morphology of some fruit species using SEM on apple, pear, quince, apricot, plum, peach, almond, chestnut, walnut, pomegranate, and persimmon cultivars. The surface features, as well as length, width, and P/E ratio of pollen were observed. Pollen length and width changed with investigated species and cultivars; the longest and widest pollen grains were detected in peach and walnut, respectively. The pollen, according to P/E ratio, was prolate, suboblate, and perprolate based on the material. Regarding the surface features, the ornamentation of pear, quince, plum, peach, and almond pollen was striate and tectum perforatum, whereas that of apple, chestnut, apricot and pomegranate was striate, rugulate, and tectum imperforatum. Walnut and persimmon had special surface characteristics.

Key Words: Pollen, SEM, pome fruits, stone fruits, nuts, Mediterranean fruits

Bazı Meyve Türlerinin Çiçek Tozu Morfolojisi Üzerine Araştırmalar

Özet: Morfolojik karakterler, bitki materyalinin tanımlanması amacıyla kullanılmaktadır. Çiçek tozu morfolojisi de bu konudaki önemli parametrelerden biridir. Bu durum, Taramalı Elektron Mikroskobun ayrıntılı çalışmalarda kullanılması ile önem kazanmıştır. Bitki materyali tanımlama aracı geliştirme düşüncesiyle yola çıkılarak, bu araştırma, elma, armut, ayva, kayısı, erik, şeftali, badem, kestane, ceviz, nar ve Trabzon hurması türlerine dahil çeşitlerin çiçek tozu morfolojik karakterlerinin, Taramalı Elektron Mikroskop kullanılarak belirlenmesi amacıyla gerçekleştirilmiştir. Çiçek tozlarında, yüzey özellikleri, uzunluk, genişlik ve P/E oranı belirlenmiştir. Çiçek tozu uzunluk ve genişliği tür ve çeşitlere gore değişiklik göstermiş olup, en uzun ve en geniş çiçek tozları sırası ile şeftali ve ceviz türlerine ait olmuştur. P/E oranına göre çiçek tozu şekilleri prolate, suboblate ve perprolate olarak saptanmıştır. Yüzey özellikleri açısından, armut, ayva, erik, şeftali ve badem türleri striate ve delikli iken, elma, kestane, kayısı ve narda striate, rugulate ve deliksizdir. Ceviz ve Trabzon hurması ise spesifik bazı yüzey özelliklerine sahip olmuştur.

Anahtar Sözcükler: Çiçek tozu, taramalı elektron mikroskop, yumuşak çekirdekliler, sert çekirdekliler, sert kabuklular, Akdeniz meyveleri

Introduction

Pollen, which is a male gametophyte of plants, can preserve its structure for hundreds of years. Pollen consists of exine and intine layers. The exine layer is an outer wall of the pollen and composed of a very tough sporopollenin substance. The intine layer is an inner wall made up of cellulose. The exine layer contains pores and ridges that are elongated and parallel elements on the surface of pollen. The ornamentation of the outer wall can appear meshed, granular, grooved, spined, striated, or smooth. Although pollen shape varies, a great majority of pollen is basically spherical or oval. Pollen size generally ranges from 15 to 100 microns (Robertson, 2008).

For the identification of plant material, morphological characteristics such as plant vigor and leaf, flower, and fruit parameters could be used. Additionally, since pollen

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has its own unique set of characteristics such as size, exine structure, and number and size of pores on the exine of pollen, these features can be taken into account to distinguish species and cultivars. Using pollen morphology in identification has become more important since the advent of the scanning electron microscope (SEM) (Fogle, 1977a, 1977b; Maas, 1977; Cargnello et al., 1980; Galletta, 1983; Hebda et al., 1988; Merev, 1996; Jones, 2001; Perveen and Qaiser, 2003).

The main objective of this study was to determine the pollen morphology of some important fruit species by SEM in order to develop a plant material identification tool.

Materials and Methods

Materials

This research was carried out on pollen samples obtained from productive trees at Ege University Ödemiş Vocational Training School and orchards in Ödemiş, İzmir. The species and cultivars investigated in the current study are listed in Table 1 based upon the specific family affiliation.

Methods

The flower buds of species belonging to the families *Rosaceae*, *Ebenaceae*, and *Punicaceae* were taken at the balloon stage in order to collect pollen; sepals and petals of the buds were removed, and anthers were isolated, put into petri dishes, and kept at room temperature for 18-20 h. In chestnuts and walnuts, catkins were collected, put into petri dishes, and kept at room temperature. Afterwards, the pollen was sprinkled on the surface. The pollen was further dried at room temperature for 6-8 h and put into brown glass bottles with silica gel. Samples were stored in a refrigerator at +4 °C until examined.

In order to investigate the pollen structure, a JEOL-JFM-5200 (Tokyo, Japan) SEM was used. This investigation was carried out at Ege University Faculty of Dentistry. To prevent pollen dispersion due to pressure, double-sided sticky tape was stuck on the SEM stubs. The pollen was scattered around the surface of the strips. After that, the pollen was coated with gold with 200 Å thickness in a vacuum evaporator under certain pressure. The pollen was examined and afterwards photographed. The evaluation of pollen was made using those photographs (Fogle, 1977a; Sağlam, 2004). Both shape and colpus structure, as well as ectoaperture, ornamentation, and perforation of the pollen were identified.

For each cultivar, 10 pollen grains were used to determine the pollen size. Both width and length were measured in microns, and P/E (polar axis/equatorial diameter) ratio was determined using the length/width formula (Erdtman, 1943). According to this ratio, pollen shape was identified as suboblate (0.75-0.88), oblate spheroidal (0.88-1.00), subprolate (1.14-1.33) (Erdtman, 1952), prolate (1.33-2.00), and perprolate (>2.00) (Erdtman, 1943).

Table 1. The species and cultivars examined in the study.

	Species	Variety		Species	Variety
Pome fruits (Rosaceae)	Pear	Ankara Dr. Jules Guyot	Stone fruits (Rosaceae)	Apricot	Tokaloğlu Tyrinthe
	Apple	Starkrimson Granny Smith		Plum	Aynalı Formosa
	Quince	Limon Ekmek		Peach	Cardinal Monroe
Nuts	Almond (Rosaceae)	Texas Marcona	Mediterranean fruits	Pomegranate (Punicaceae)	Hicaz 26/3 Çekirdeksiz
	Chestnut (Fagaceae)	Osmanoğlu Seyrekdiken		Persimmon (Ebenaceae)	Pollinator Type 1 Pollinator Type 2
	Walnut (Juglandaceae)	Kaplan 86 Şebin			

Pollen can be described as tricolporate or pantocolporate in terms of colpus. Pollen with 3 ectocolpi, 3 compound apertures, or 3 pores is called tricolporate (Iversen and Troels-Smith, 1950). In contrast, pollen with apertures spread over the surface sometimes forming a regular pattern is called pantocolporate (Erdtman and Vishnu-Mittre, 1956).

The ectoaperture is an aperture in the outer layer of the sporoderm (Van Campo, 1958). Ectoapertures can show up as slits (Hebda and Chinnappa, 1990) or as pores (Jackson, 1928; Wodehouse, 1935).

Ornamentation is a general term that is used for describing the composition of features (Potonié, 1934). The ornamentation could be striate when elongated and generally parallel elements are separated by grooves, rugulate when elongated elements are more than 1 µ long and are arranged in an irregular pattern (Iversen and Troels-Smith, 1950), as well as microechinate with features less than 1 µ on the pollen surface (Hoen, 1999), or the surface can be overall smooth.

The perforation is a hole less than 1 µ in diameter generally situated in the tectum (Iversen and Troels-Smith, 1950). If there are perforations on the surface of pollen, it is described as tectum perforatum (Iversen and Troels-Smith, 1950). On the other hand, if there are no perforations on the pollen, it is called tectum imperforatum (Walker and Doyle, 1975). In order to find out the number of perforations (= puncta, <1 µ) on the exine surface, perforations in 1 μ^2 were counted in 5 different locations on the surface. In order to calculate the average diameter of the perforations, 10 different perforations were measured.

The evaluation was made based upon the above-mentioned details and the mean values of the investigated parameters were also taken into account.

Results

The length, width, P/E ratio, number of perforates per μ^2, and diameter of perforates (µ) of pollen of the cultivars studied are shown in Table 2.

Pome fruits

Apple

The pollen of Starkrimson and Granny Smith cultivars was both cylindrical and tricolporate. Ectoapertures were slits. In both cultivars, ridges with large grooves were observed. The surface of the apple pollen was striate but not perforate (Figure 1a and b). Thus, pollen was described as tectum imperforatum. Ridges of Granny Smith pollen were parallel from one pole to another (Figure 1c). In Starkrimson, ridges with deep grooves were more irregular than in the other cultivar (Figure 1d). In both apple cultivars, the pollen was prolate.

Pear

The pollen of 2 different cultivars of pears, Ankara and Dr. Jules Guyot, was cylindrical and tricolporate. Ectoapertures were slits. Perforations smaller than 1 µ diameter were irregularly distributed on the tectum. The Ankara cultivar had many more perforations than did Dr. Jules Guyot. In both cultivars, ridges could be seen on the surface, and their ornamentations were striate (Figure 1e and f). Ridges were parallel to ectoapertures through one pole to another and perforates were located in the grooves in Ankara (Figure 1g). In Dr. Jules Guyot, they were less indented and curled through poles resembling fingerprints. Moreover, this cultivar's perforations were located on or in the grooves (Figure 1h). The pollen of these cultivars was prolate.

Quince

Similar to apple and pear, quince pollen was cylindrical and tricolporate. Ectoapertures were slits. Ridges on the surface were less clear than those in apple and pear pollen; ornamentation was striate (Figure 2a and b). Ridges resembled fingerprints in the Limon variety, whereas they were irregular in Ekmek (Figure 2c and d). Quince pollen was of the tectum perforatum type. In both cultivars, perforations were scattered irregularly throughout the surface of the pollen and were located on and in the grooves. Perforations on the surface of Ekmek cultivar's pollen were bigger than those on Limon (Figure 2c and d). The pollen of the Limon cultivar was identified as perprolate and that of the Ekmek cultivar as prolate.

Stone fruits

Apricot

The apricot pollen was more tumid and circular compared to the other pollen. There were 3 ectoapertures (tricolporate) and they were all slits. The pollen of the Tokaloğlu cultivar had verrucose-shaped outgrowths in the middle of ectoapertures that could not be seen in the Tyhrinte cultivar (Figure 2e and f). In both

Table 2. The measured pollen characteristics in different species and cultivars.

Species and Cultivars	Pollen Length(=P) (µ)		Pollen Width (=E) (µ)		P/E Ratio	Number of Perforates in μ^2	Diameter of Perforates (µ)
	Variation Range	Mean Value	Variation Range	Mean Value			
POME FRUITS							
Apple (Starkrimson)	40.0- 45.7	43.08	20.0-27.2	23.14	1.90	T.I.	T.I.
Apple (Granny Smith)	40.0- 43.3	41.80	20.0-22.7	21.30	1.96	T.I.	T.I.
Apple (Mean)	40.0-45.7	42.80	20.0-27.2	22.20	1.93	T.I.	T.I.
Pear (Ankara)	37.1-41.5	39.40	21.4-24.3	22.90	1.72	3.20	0.25
Pear (Dr. Jules Guyot)	39.4-42.2	40.60	21.1-25.7	22.60	1.80	1.20	0.25
Pear (Mean)	37.1-42.2	40.00	21.1-25.7	22.75	1.76	2.20	0.25
Quince (Limon)	42.8-57.1	51.70	21.4-28.6	25.10	2.06	3.60	0.22
Quince (Ekmek)	48.9-54.3	51.60	24.3-30.5	27.60	1.87	2.90	0.25
Quince (Mean)	42.8-57.1	51.65	21.4-30.5	26.35	1.97	3.25	0.24
STONE FRUITS							
Apricot (Tokaloğlu)	28.6-31.4	30.60	28.6-35.7	32.50	0.94	T.I.	T.I.
Apricot (Tyrinthe)	31.4-35.7	33.60	25.0-28.6	27.70	1.21	T.I.	T.I.
Apricot (Mean)	28.6-35.7	32.10	25.0-35.7	30.10	1.08	T.I.	T.I.
Peach (Cardinal)	46.9-62.5	57.16	25.0-34.4	29.80	1.92	2.50	0.48
Peach (Monroe)	59.4-62.5	61.70	37.5- 43.8	40.60	1.52	1.00	0.40
Peach (Mean)	46.9-62.5	59.45	25.0-43.8	35.20	1.72	1.75	0.44
Plum (Aynalı)	38.6-45.7	41.80	20.0- 25.7	23.40	1.79	2.00	0.25
Plum (Formosa)	37.1- 44.3	40.30	20.0-24.3	22.50	1.79	T.I.	T.I.
Plum (Mean)	37.1-45.7	41.05	20.0-25.7	22.95	1.79	2.00	0.25
TREE NUTS							
Almond (Texas)	48.6-55.7	52.20	25.7-31.4	27.40	1.91	1.20	0.28
Almond (Marcona)	48.5- 54.9	51.98	26.3-29.8	27.37	1.90	1.18	0.28
Almond (Mean)	48.5-55.7	52.09	25.7-31.4	27.39	1.91	1.19	0.28
Chestnut (Osmanoğlu)	16.7-17.8	17.25	6.5-9.2	7.60	2.27	T.I.	T.I.
Chestnut (Seyrekdiken)	11.3-18.3	15.20	8.0-9.2	8.70	1.75	T.I.	T.I.
Chestnut (Mean)	11.3-18.3	16.25	6.5-9.2	8.15	2.02	T.I.	T.I.
Walnut (Kaplan 86)	26.3-30.5	28.40	34.7-43.1	37.70	0.75	T.I.	T.I.
Walnut (Şebin)	26.7-30.1	28.30	35.3-42.8	37.20	0.76	T.I.	T.I.
Walnut (Mean)	26.3-30.5	28.35	34.7-43.1	37.45	0.76	T.I.	T.I.
MEDITERRANEAN FRUITS							
Persimmon (Pollinator 1)	51.6-53.7	52.64	29.0-29.5	29.40	1.79	T.I.	T.I.
Persimmon (Pollinator 2)	50.8-54.2	52.60	28.4-29.6	28.98	1.82	T.I.	T.I.
Persimmon (Mean)	50.8-54.2	52.62	28.4-29.6	29.19	1.81	T.I.	T.I.
Pomegranate (Hicaz)	27.2-28.3	27.70	15.5-17.2	16.60	1.67	T.I.	T.I.
Pomegranate (26/3 Çekirdeksiz)	26.6-27.9	27.82	15.2- 17.7	16.46	1.69	T.I.	T.I.
Pomegranate (Mean)	26.6-28.3	27.76	15.2-17.7	16.53	1.68	T.I.	T.I.

T.I. : No perforates on the surface of the pollen (tectum imperforatum)

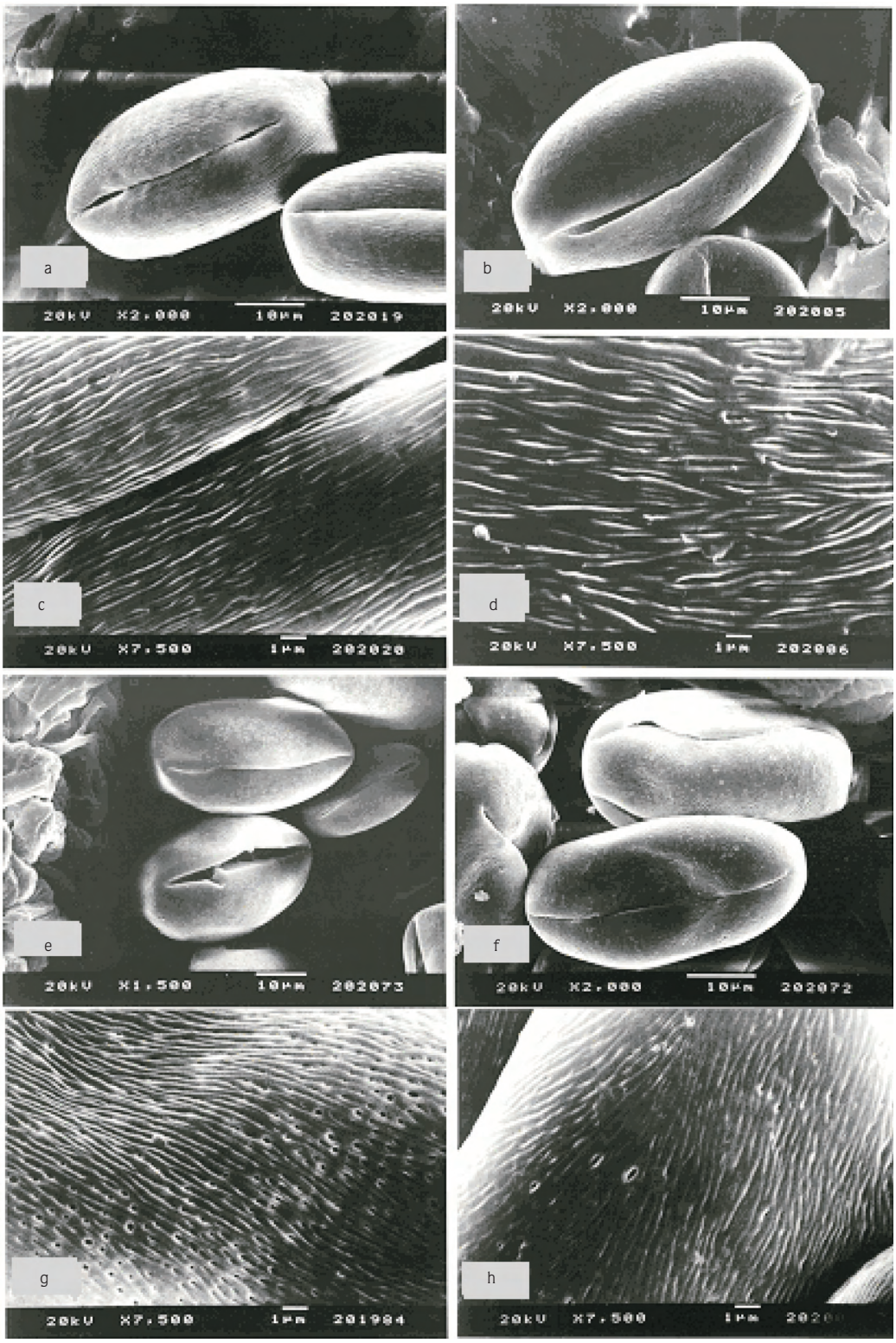

Figure 1. (a) Apple cv. Granny Smith. (b) Apple cv. Starkrimson. (c) Apple cv. Granny Smith. (d) Apple cv. Starkrimson. (e) Pear cv. Ankara. (f) Pear cv. Dr. Jules Guyot. (g) Pear cv. Ankara. (f) Pear cv. Dr. Jules Guyot.

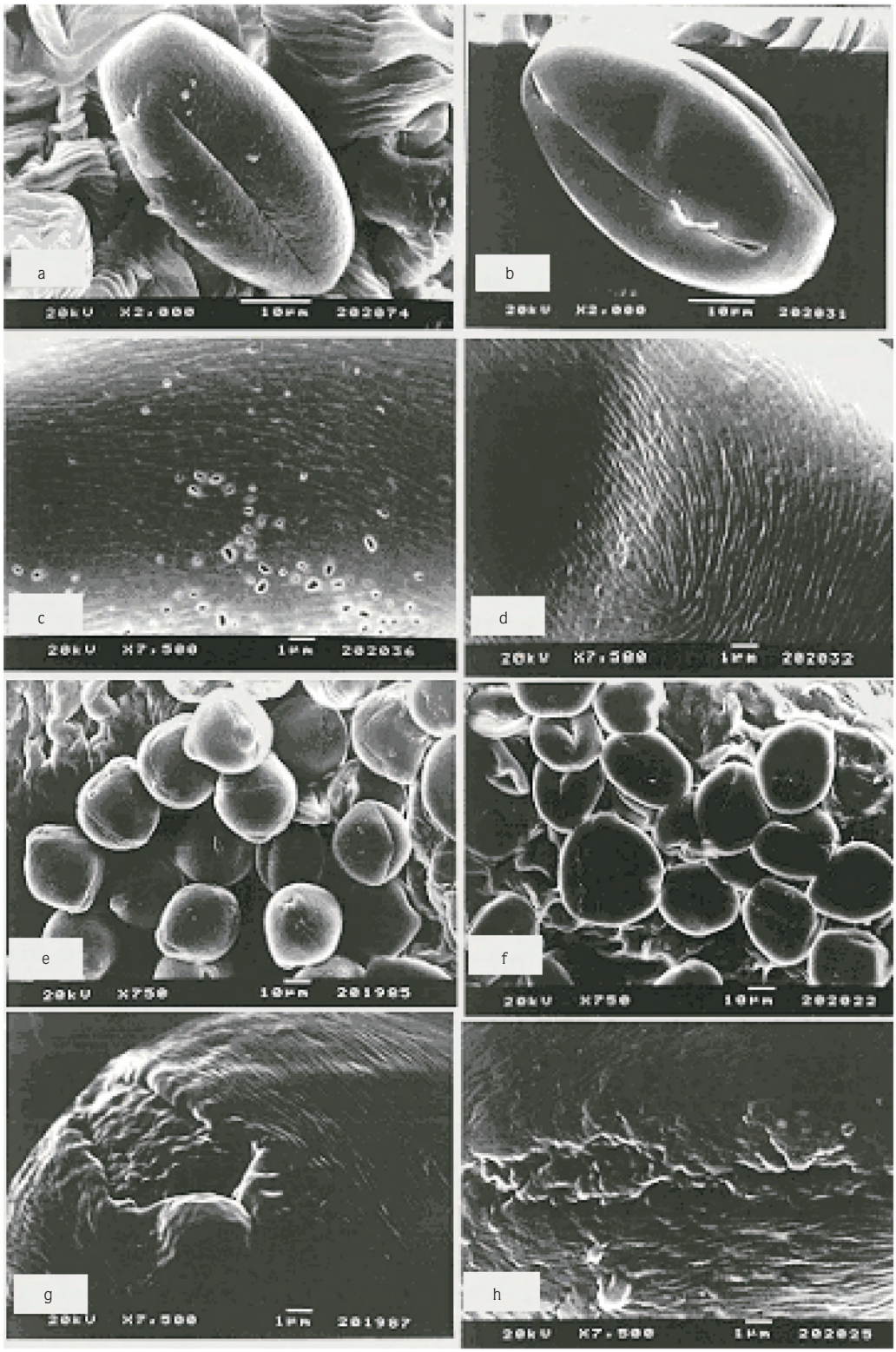

Figure 2. (a) Quince cv. Ekmek. (b) Quince cv. Limon. (c) Quince cv. Ekmek. (d) Quince cv. Limon. (e) Apricot cv. Tokaloğlu. (f) Apricot cv. Tyrinthe. (g) Apricot cv. Tokaloğlu. (h) Apricot cv. Tyrinthe.

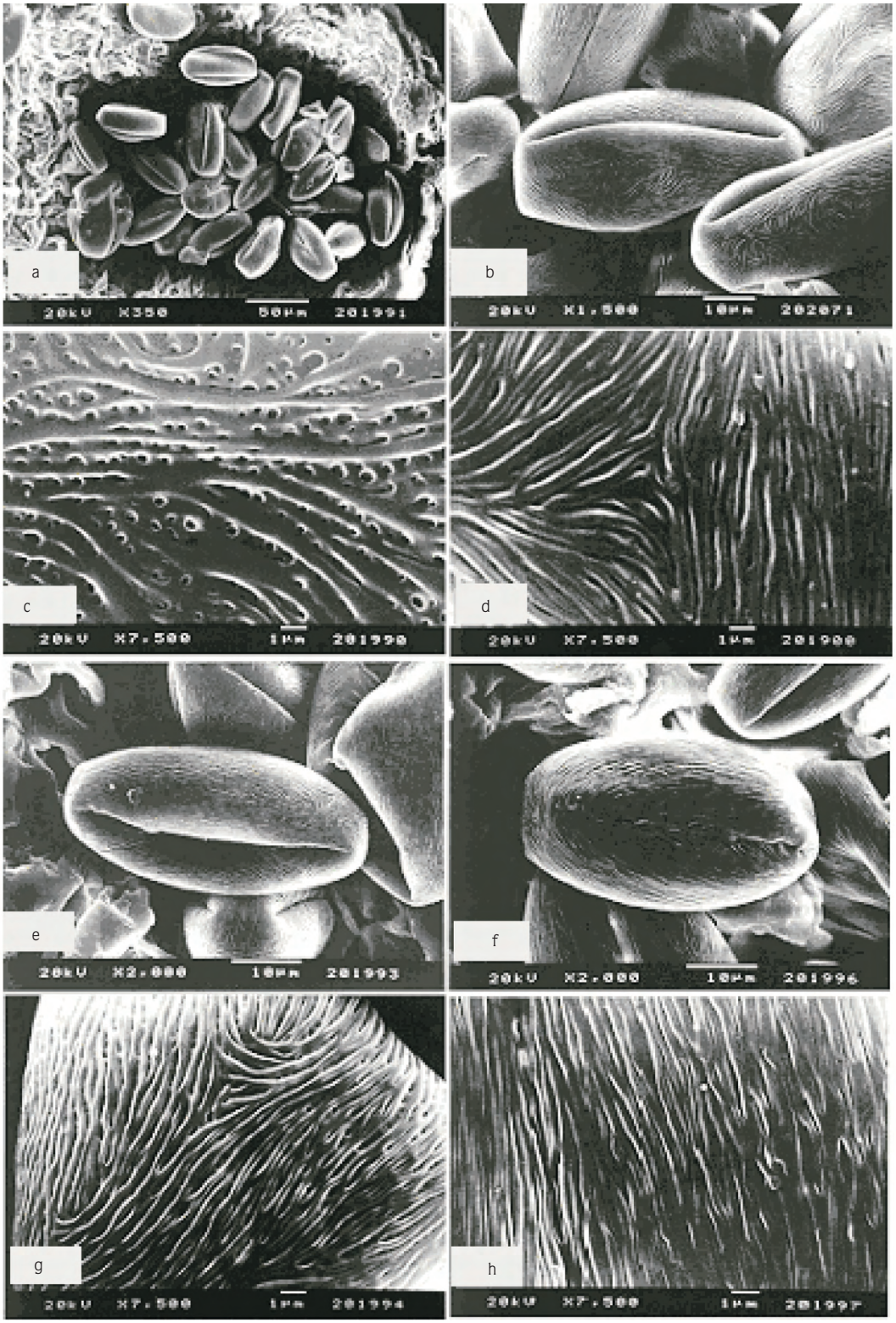

Figure 3. (a) Peach pollen. (b) Peach pollen. (c) Peach cv. Cardinal. (d) Peach cv. Monroe. (e) Plum cv. Aynalı. (f) Plum cv. Formosa. (g) Plum cv. Aynalı. (h) Plum cv. Formosa.

cultivars there were no perforations on the exine surface. There were thick ridges parallel through the poles on the pollen surface. Their ornamentation was striate. These ridges were more regular in the Tokaloğlu cultivar (Figure 2g). In both cultivars, large or small verrucose shapes on different parts of the surface were observed, but the Tyhrinte cultivar had many more of these outgrowths (Figure 2h).

The pollen was spheroidal and subprolate in the Tokaloğlu and Tyhrinte cultivars, respectively.

Peach

The peach pollen was cylindrical and tricolporate, and ectoapertures were slits. The pollen size was variable (Figure 3a and b). There were perforations smaller than 1 µ diameter on the tectum of both cultivars. Perforations on the surface of the Cardinal cultivar's pollen were clearer than those on the Monroe cultivar. A sieve-like surface was clearly observed on Cardinal cultivar's pollen. In both cultivars, the ornamentation was striate, ridges were irregular on the surface of pollen and perforations were located in the grooves (Figure 3c and d). Both peach cultivars had prolate pollen when considering both its length and width.

Plum

As with apricot and peach, plum cultivars had pollen that was cylindrical, with 3 lobes, and the ectoapertures were slits. Similar to peach pollen, the plum pollen sizes were varied. There were thick ridges with large grooves and the ornamentation was striate. The Aynalı cultivar's pollen was tectum perforatum, while the Formosa cultivar's pollen was tectum imperforatum (Figure 3e and f). Perforations were located in the grooves, and ridges were arranged like fingerprints throughout the poles of pollen in the Aynalı cultivar (Figure 3g). In the Formosa cultivar's pollen, ridges on the surface were much more regular than those on the surface of the other cultivar (Figure 3h). The pollen was prolate in both plum cultivars.

Nuts

Almond

The almond pollen was cylindrical and tricolporate and had 3 slitted ectoapertures. There were ridges and perforations on the surface of pollen. The ornamentation was striate just like on the other *Rosaceae* species. The ridges on the pollen of both cultivars were clear, regularly shaped, and parallel from one pole to another. Perforations were located in the grooves and no difference was observed on the surfaces of either investigated cultivar. The pollen was prolate in both cultivars.

Chestnut

The pollen of chestnut species (*Fagaceae*) was both cylindrical and tricolporate. The ectoapertures were slitted. The chestnut had the smallest pollen among the investigated species. The pollen surface was rugulate. Pole surfaces of the pollen were smooth. The pollen of both cultivars had warty-shaped outgrowths in the middle of ectoapertures. There were no observable differences between the 2 cultivars of chestnut: Osmanoğlu and Seyrekdiken. By analyzing the length and width data, Osmanoğlu was identified to have perprolate pollen whereas Seyrekdiken had prolate pollen.

Walnut

Among all investigated species and cultivars, only walnut pollen was pantocolporate and nearly circular (spherical). There were pores spread all over the surface of pollen of each cultivar, and so the pollen was identified as pantoapertured. The pollen surface was microechinate. There was no difference between the 2 walnut cultivars, and the pollen of both cultivars was suboblate.

Mediterranean fruits

Persimmon

The persimmon pollen was cylindrical and had 3 ectoapertures (tricolporate). These ectoapertures were slitted. The selected cultivars were grown as pollinizers in persimmon orchards. Persimmon cultivar pollen had smooth surfaces and did not have any ridges or perforations on it. This was very specific only for this species among all of the investigated ones. No difference was observed among the 2 pollinizer cultivars. The pollen in both cultivars was prolate.

Pomegranate

The pollen of pomegranate was cylindrical and had 3 slitted ectoapertures (tricolporate). The ornamentation was rugulate. There were elongated exine elements over 1 µ in size arranged in an irregular pattern. There were no perforations on the surface of either cultivar's pollen. The pollen was tectum imperforatum. There was no difference between the Hicaz cultivar and the 26/3 Çekirdeksiz cultivar. The pollen was prolate in both pomegranate cultivars.

Discussion

Among the investigated species, peach ranked first in terms of pollen length, followed by persimmon and almond. Chestnut, pomegranate, and walnut were ranked in the last positions according to this parameter. For pollen width, the highest value was determined in walnut, followed by peach and apricot. Chestnut, pomegranate, and apple pollen had the lowest values (Table 2).

In the general evaluation of pollen surfaces, it was found that the ornamentation of pear, quince, plum, peach, and almond pollen was striate and tectum perforatum, whereas that of apple, chestnut, apricot, and pomegranate was striate, rugulate, and tectum imperforatum. The walnut pollen had a special surface characteristic defined as microechinate. Only persimmon did not have any ridges or perforations, leading to a smooth surface.

The average pollen length and width in Rosaceae species were 32.10-59.45 µ and 22.20-35.20 µ, respectively. The pollen was monad and tricolporate, with slitted ectoapertures, as well as medium sized, prolate (except for in apricot); the ornamentation was striate, either tectum perforatum or imperforatum. This research was substantiated through similar results obtained for *Malus sylvestris*, *Prunus cerasifera*, *Prunus dulcis*, *Prunus spinosa*, and *Cydonia oblonga* species of Rosaceae (Halbritter, 2000a, 2000b, 2000c, 2000d; Halbritter and Schneider, 2000).

The average pollen length and width in chestnut were 16.25 µ and 8.15 µ, respectively. The pollen was monad, tricolporate, perprolate, the ornamentation rugulate and tectum imperforatum. In other research, similar pollen size of this species was determined (10-25 µ) by Halbritter and Sam (2000a), and pollen was identified as monad, tricolporate, and tectum imperforatum with rugulate ornamentation.

The average length and width of walnut pollen were 37.45 µ and 28.35 µ, respectively. The pollen was medium sized, monad, panto-aperturate, suboblate, and microechinate. Similar data were obtained by Halbritter and Sam (2000b), who reported that walnut pollen is medium-sized (26-50 µ), oblate, monad, panto-aperturate, and microechinate.

The pomegranate had small pollen (16.53-27.76 µ) and was evaluated as monad, tricolporate, prolate, with rugulate ornamentation, and tectum imperforatum. Similar results were obtained by other researchers. According to their data, the pollen was shed as single grains (Watson and Dallwitz, 2008).

In persimmon, the average length of pollen was 52.62 µ and the width was 29.19 µ. The pollen was medium-sized, monad, tricolporate, with slitted ectoapertures, as well as prolate and tectum imperforatum with a smooth surface.

In the current study, the pollen characteristics of fruit and nut species were investigated by SEM, and some differences in relation to species and cultivars were detected in terms of pollen surfaces and sizes. The surface features differed in the examined cultivars of apple, pear, quince, apricot, peach, and plum species. In contrast, the surface features were the same in the investigated cultivars of almond, chestnut, walnut, persimmon, and pomegranate. P/E ratio was different in cultivars of quince, apricot, and chestnut species, but similar in the rest of them. Confirming these data, similar results were obtained by different researchers (Fogle, 1977b; Westwood and Challice, 1978; Davarynejad et al., 1995). Differences among Turkish indigenous grape cultivars were found in terms of pollen surface characteristics and sizes (Marasalı et al., 2005). Consequently, some morphological characteristics of pollen, such as surface features, number and distribution of perforations, and pollen sizes, could be used to distinguish cultivars and species. According to these results, pollen characteristics are of importance as far as general description is concerned. The method could be useful at the first stage of identification studies because it is simpler and more economical than the other more detailed methods. This information is expected to be useful for plant breeders or for gene bank curators who may need to know features of pollen for general description studies.

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