

Pollen characteristics of some grape cultivars (*Vitis vinifera* L.)

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Abstract

Pollen morphology is an important parameter. In this study, the pollen morphology of selected in 10 grape cultivars were examined by SEM (scanning electron microscopy). The pollen surface features were observed, such as length, width, and P/E ratio of pollen. The pollen differed in some microstructural characteristic. Pollen width exhibited significant according to the varieties (10.12-22.44 µm). Similarly, the statistical difference occurred among the ten *Vitis* genotypes in terms of mean pollen length (16.26-29.91 µm). P/E ratio determined to the varieties (1.08-2.55 µm) and, grape varieties have the longest pollen, in general. The without furrows are surrounded in exine were determined in cultivars of “Alicante Boushet”, “Cardinal”, “Syrah”. Areolat pollen was determined in among other cultivars. Depending on the cultivars there was a statistical difference from the point of pore diameter. Pollen viability was determined between 11.75 % and 84.25 % in TTC tests. The present research is a contribution to amore detailed analysis of grapevine cultivars.

Keywords: Palynological, Pollen, Pollen viability, Pollen germination, Scanning electron microscope (SEM), TTC test, Classification

Introduction

Pollen has hereditary properties that determine the genotype of plants. Pollen morphology confirms phylogenetic relationships among genera, species and varieties. Thus, it is used in systematic studies due to similarity and diversity of pollen. The morphology of pollen can be examined in detail via the scanning electron microscope (Tanaka et al., 2004).

On the basis of surface ornamentation and pollen grain dimensions, different classifications have been made on various plant species (Erdtman, 1952; Faegri and Iversen, 1989; Hyde and Adams, 1958; Wodehouse, 1935). Studies in grapevines have mainly focused on cultivars. In the description of the pollen exine microrelief, separate elements, polar and equatorial axis, mesocolpium, apocolpium, and length and width of the colps are used (Roytchev, 1995). For example, Uzun and Ilter (1987) and Kharitonashvili et al. (1989) studied

pollen grains in different types of flowers of *Vitis vinifera* L., using SEM. Ahmedullah (1983) characterized different grape cultivars based on pollen morphology. Martens et al. (1989) studied pollen size variability within genotypes of *Vitis*, Ben Slimane (1990) characterized 30 grapevine varieties based on pollen size, Roytchev et al. (1994) obtained information on the ultrastructure of exine surface in 27 Bulgarian and foreign seedless grape cultivars and Gallardo et al. (2009) studied fourteen Spanish *Vitis vinifera* L. subsp. *silvestris* populations. Palynology has presented considerable opportunities for newly released hybrid varieties identification in grapevines (Marasali et al. 2005). Jovanovic-Cvetkovic et al. (2016) analysed the pollen morphology of indigenous cvs. Žilavka and Blatina to determine their morphological specificities.

The objective of the present study was to classify the ten

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grape cultivars according to the shape and microrelief of pollen grains and to establish the possibilities for using the parameters of the different apertures as classification indices using SEM.

Materials and methods

Pollen samples:

This study was carried with pollen from ten different of *Vitis vinifera* L. (Table 1) are located at the Department of Horticulture, Agriculture Faculty, Ege University (38° 27' 15.8652" ve 27° 13' 32.5272"). The flower clusters were isolated and these inflorescences were collected in the morning hours at the beginning of the blooming period (when the calyptas fall from the flower a cloud of pollen is released from the anthers of the stamens which move away from the pistil) of Eichorn and Lorenz (1977) classification. Since bloom may progress for several days over a vine and an individual cluster, one must estimate the percentage of cap fall to designate the stage of development. In the varieties examined in the study, full bloom occurred between 25 May- 8 June. Samples from "Cardinal" were collected on 25 May, "Foça Karası" and "Yuvarlak Çekirdeksiz" on June 1, "Alicante Bouschet", "Cinsaut", "Mahrabası", "Malbec", "Syrah" on June 5, "Alphonse Lavallee" and "Italia" on June 8, respectively. The pollen was collected by cutting flowers and brushing the anthers and pollen into an Eppendorf tube using a soft brush (Gökbayrak and Engin, 2016). The study materials were stored a refrigerator in controlled conditions until analysis (Storey, 1975).

Dry pollen was sputter-coated (Leica model) with 10 µm of gold-palladium, for this pollen grains were air dried for SEM investigations. Pollen grains were measured directly on the screen of the scanning electron microscope. Each of the tested samples were observed with Thermo Scientific Apreo S model scanning electron microscope were photographed at 10000 x for whole grain (Central Research Test and Analysis Laboratory Application and Research Center (EGE MATAL) for help on SEM operation). The pollen length, width, length/

width ratio and pore diameter, distance between pori and colpi length were measured on 10 pollen grains for each genotypes. The pollen shape was stated by considering the length/width ratio (Erdman, 1952). The types of aperture found in pollen were described by Wang et al. (2014). The polar and equatorial axes, the pollen grain equatorial diameter relationship were determined by Erdman (1952) and La-Serna Ramos (1996) was used for ultra morphological descriptions of the pollen grains.

Pollen viability:

1% of TTC was used for the viability capacity of pollens in this study (Norton, 1966). The pollen samples were carried out through eight replications in four fields chosen randomly on each microscope slide. In TTC test, they were classified into dark red pollen grains, light red pollen or colourless pollens according to their colours. Pollen were considered as active, semi-active, and lifeless, respectively (Kelen and Demirtaş, 2003).

Pollen germination:

To calculate the *in vitro* germination percentage of 10 grape cultivars, a medium containing 10 mg of boric acid per ml, 20 % sucrose, and 0.6 % agar was used. Pollen grains were placed agar in petri dishes containing the germination medium that were maintained at 30°C in the dark for 24 h (a thermos controlled dryer). To determine the germination percentage, 3 fields per sample (each one containing around 100 pollen grains) were counted using a light microscope (Kelen and Demirtaş 2003).

The data were subjected to analysis of variance using SPSS 20 statistical package program. The mean, minimum, maximum, and standard error values of the properties were found out. The relationship among these values was revealed by conducting Pearson's correlation analysis. Cluster analysis (CA) was also realized and indicated by dendrogram (Maraslı et al., 2005).

Table 1. List of the cultivars studied

Cultivars	Type	Cultivars	Type
<i>Vitis vinifera</i> L. "Alphonse Lavallee"	T, S	<i>Vitis vinifera</i> L. "Italia"	T, S
<i>Vitis vinifera</i> L. "Alicante Bouschet"	W, S	<i>Vitis vinifera</i> L. "Mahrabası"	T, S
<i>Vitis vinifera</i> L. "Cardinal"	T, S	<i>Vitis vinifera</i> L. "Malbec"	W, S
<i>Vitis vinifera</i> L. "Cinsault"	W, S	<i>Vitis vinifera</i> L. "Syrah"	W, S
<i>Vitis vinifera</i> L. "Foça Karası"	W, S	<i>Vitis vinifera</i> L. "Yuvarlak Çekirdeksiz"	T, SE

Abbreviations: T: table grape, W: Wine grape, S: Seeded, SE: Seedless grape

Results

Given the characteristics of ten grape cultivars, a general description was established for all, according to the values of the various parameters corresponding to the max. and min. records. The statistical difference appeared in terms of the pollen grains (length, width and length/width ratio of pollen) (Table 2 and Table 3).

Mean pollen width differed statistically significant

according to the varieties. Thus, the highest mean values for this feature was in "Mahrabası" (22.44 µm, a) and the lowest mean values for this pollen width were found in "Syrah" (10.54 µm, f) and "Alicante Bouschet" (10.12 µm, f) varieties, respectively. Pollen length ranged from 29.91 µm (a) "Alphonse Lavallee" to 16.26 µm (e) "Foça Karası" (Table 2).

On the other hand, when the pollen is examined in terms of symmetry and shape, the length/width ratio ranged from 2.55

μm “Alicante Bouschet” to 1.08 “Foça Karası” (Table 2). The morphological description of pollen was made using Erdman (1952)’s terminology. Among the grape varieties examined four different shapes. The pollen grains were prolate-spheroidal “Foça Karası”, subprolate “Mahrabası”, perprolate (Alicante Bouschet, Syrah, Cardinal, Italia) and prolate (Alphonse Lavallee, Malbec, Cinsault, Yuvarlak Çekirdeksiz) (Table 6).

According to aperture, typically two types were observed. Among the grape varieties examined, it was determined that there was no diaphragm opening in the pollen of a group. Inaperturate pollen grains were observed in some cultivars such as “Foça Karası”, “Mahrabası”, whereas “Alphonse Lavallee”, “Cinsaut”, “Alicante Bouschet”, “Malbec”, “Italia”, “Syrah” “Cardinal”, “Yuvarlak Çekirdeksiz” were tricolporate (Figure 1).

Circular openings were detected on the pollen grains and such grains are called porate. The pollen had circular apertures on the exine surface, these are not uniformly distributed and the pollen grains were said pantoporate. The pollen grains surface has elongated or furrow-like apertures. These were called colp. Also, the circular apertures on the pollen has circular apertures on the exine surface, they were called pori. The pollen shape and exine patterns of the studied varieties were given in SEM images. There were statistical differences in terms of these properties. For pore length, “Cinsaut” (397.45, a) located at the first group, while the “Alphonse Lavallee” (112.40, d) was the last group. Thus, pore width differed statistically significant according to the varieties. For this value, the “Cardinal” (245.68, a), variety was the first group, among the varieties examined, “Syrah” and “Alphonse Lavallee” were the smallest diameter of the pore width and, “Syrah” (97.06, e) and “Alphonse Lavallee” (102.32, e) located at the last group. In terms of this feature, it was found in different statistical groups in other varieties (Table 3).

Pollen of “Alicante Bouschet”, “Syrah”, “Cardinal” cultivars did not furrows. Areolat is surrounded in exine was observed in “Foça Karası”, “Mahrabası”, “Alphonse Lavallee”, “Cinsaut”, “Malbec”, “Italia”, “Yuvarlak Çekirdeksiz”

(Figure 2).

It was determined that to pollen germination ranged from 29.25 % and 87.25 %. In respect to pollen viability levels varied between 11.75 % and 84.25 % in TTC tests. “Cardinal”, Alicante Bouschet” and “Syrah”, cultivars were without furrows and TTC test result in these cultivars was found 84.25 %, 75.75 % and 74.25, respectively (Table 5).

The correlation coefficients of the features are shown in Table 4. Accordingly, the highest positive correlation was determined between TTC and pollen length/width ratio ($r=0.700$; $p<0.05$). From the other side, a negative correlation occurred between the pollen length/width ratio and pollen width value ($r=-0.796$; $p<0.01$) and TTC and pollen width ($r=-0.700$; $p<0.05$). The similarities or differences among the grape cultivars examined with Pearson correlation coefficients showed a correlation with those examined with TTC test in terms of examined characteristics.

Cluster analysis was used to determine the degree of similarity of grape cultivars, is located in Figure 4 as dendograms. Consequently, the cultivars were classified under two main groups. “Alicante Bouschet”, “Yuvarlak Çekirdeksiz”, “Foça Karası”, “Mahrabası”, “Malbec”, “Cinsaut”, “Cardinal” were included in the first group while “Italia”, “Syrah”, “Alphonse Lavallee”, were collected in the second group. First and second groups divided into different sub-groups.

Discussion

A number of palynological investigations into cultivated *Vitis* varieties have shown that pollen shape and P/E ratio change from one sample to another (Reille, 1966; Roytchev, 1997; Cabello et al., 1994). Our results appeared that the ten cultivars of *V. vinifera* L. examined were differences in terms of pollen morphology. There were difference in the size (pollen width, pollen length), shape of pollen grains, pore on pollen surface and pollen ornamentation.

There were significantly differences in pollen width grains sizes in the cultivars studied. The length of pollen grains ranged from 16.26 μm to 29.91 μm (Table 2).

Table 2. Morphological characteristic of pollen of grape cultivars (μm)

Cultivars	Pollen Size											
	Pollen width (μm)				Pollen length (μm)				Length/width ratio			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Alphonse Lavallee	14,50	16,76	15,66 cd	0,75	28,28	32,32	29,91 a	1,64	1,75	2,08	1,91 cd	0,12
Alicante Bouschet	8,18	13,07	10,12 f	1,49	21,43	29,02	25,43 c	2,69	2,09	3,35	2,55 a	0,41
Cardinal	11,43	16,90	13,96 de	1,74	26,98	32,27	29,43 a	1,74	1,78	2,60	2,14 bc	0,27
Cinsault	14,06	17,00	15,90 c	1,18	24,34	32,86	28,42 ab	2,64	1,18	1,50	1,79 d	0,15
Foça Karası	14,17	16,89	15,04 cd	0,94	15,70	17,26	16,26 e	0,62	1,02	1,13	1,08 f	0,04
Italia	9,65	17,03	12,94 e	2,80	20,82	29,52	25,74 c	2,99	1,46	2,95	2,08 cd	0,54
Mahrabası	20,82	24,25	22,44 a	1,03	23,55	29,87	26,86 bc	2,12	1,08	1,39	1,20 ef	0,13
Malbec	9,59	18,56	12,77 e	2,56	20,44	26,11	22,59 d	1,95	1,41	2,44	1,82 d	0,36
Syrah	9,11	12,28	10,54 f	0,97	20,25	31,90	25,04 c	3,46	1,70	3,06	2,40 ab	0,39
Yuvarlak Çekirdeksiz	14,83	23,81	19,09 b	3,15	23,19	34,47	26,47 bc	3,37	1,03	1,67	1,42 e	0,26

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations

Table 3. The length and width values of pori in grape varieties (μm)

Cultivars	Pore width (μm)				Pore length (μm)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Alphonse Lavallee	84,91	135,83	102,32 e	20,13	99,46	146,52	112,40 d	20,15
Alicante Boushet	167,56	200,18	185,69 bc	16,11	194,20	280,33	217,18 bc	35,64
Cardinal	165,72	281,35	245,68 a	46,05	178,39	398,80	265,36 bc	91,58
Cinsault	124,56	176,55	158,09 cd	22,20	322,00	492,54	397,45 a	71,91
Foça Karası	141,95	260,77	196,85 abc	48,74	152,91	276,75	204,06 bc	51,24
Italia	94,86	136,80	118,63 de	21,29	159,30	305,50	219,18 bc	63,19
Mahrabaşı	149,30	315,66	220,11 ab	70,65	109,80	332,63	208,54 bc	111,45
Malbec	180,51	250,54	209,07 abc	25,73	217,20	349,90	298,82 b	52,02
Syrah	86,00	133,50	97,06 e	20,43	111,20	288,90	178,48 cd	75,82
Yuvarlak Çekirdeksiz	135,81	228,64	161,62 cd	37,84	147,30	295,60	214,60 bc	57,80

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations

Table 4. Pearson correlation coefficients among traits in cultivars

	Pollen width	Pollen length	Pollen length/width ratio	Pore width	Pore length	TTC
Pollen length	0,178					
Pollen length/width ratio	-0,796**	0,410				
Pore width	0,273	-0,175	-0,329			
Pore length	-0,009	0,046	-0,028	0,395		
TTC	-0,700*	0,021	0,700*	0,137	-0,069	
Agar in petri dishes (%)	0,176	-0,071	-0,098	0,290	-0,197	0,411

Abbreviations: * Significant at $P<0.05$, ** Significant at $P<0.01$

Table 5. Pollen viability capacity determined in the TTC tests (%) and germination rates obtained from the agar in petri dishes (%)

Cultivars	Viability				Germination			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Alphonse Lavallee	0,00	57,00	24,25 cd	23,79601	0,00	70,00	38,25 bc	29,64653
Alicante Boushet	50,00	100,00	75,75 a	21,10884	33,00	75,00	53,75 abc	17,38534
Cardinal	80,00	86,00	84,25 a	2,87228	60,00	81,00	69,25 abc	8,84590
Cinsault	0,00	23,00	11,75 d	9,42956	0,00	92,00	29,25 c	43,46167
Foça Karası	9,00	50,00	26,50 cd	18,77054	0,00	100,00	50,00 abc	57,73503
Italia	22,00	75,00	56,75 ab	23,99132	50,00	100,00	79,50 ab	21,07922
Mahrabaşı	4,00	33,00	20,25 cd	13,67175	83,00	90,00	87,25 a	2,98608
Malbec	42,00	85,00	67,00 ab	18,38478	42,00	100,00	70,75 abc	23,96351
Syrah	66,00	86,00	74,25 a	8,65544	50,00	77,00	67,00 abc	12,30176
Yuvarlak Çekirdeksiz	25,00	71,00	42,25 bc	20,12254	50,00	100,00	65,00 abc	23,80476

Table 6. Mean value for P/E ratio

	< 0.50	0.50-0.75	0.76 - 0.88	0.89 – 0.99	1.00	1.01 – 1.14	1.15 – 1.33	1.34 – 2.00	> 2.00	P/E ratio
	peroblate	oblate	suboblate	oblate-spheroidal	spherical	prolate-spheroidal	subprolate	prolate	perprolate	
Cultivars										
“Alicante Bouschet”										X (>8:4)
“Syrah”										X (8:4)
“Cardinal”										X (>8:4)
“Italia”										X (>8:4)
“Alphonse Lavallee”										X (8:4-8:6)
“Malbec”										X (8:4-8:6)
“Cinsaut”										X (8:4-8:6)
“Yuvarlak Çekirdeksiz”										X (8:4-8:6)
“Mahrabası”										X (7:8-6:8)
“Foça Karası”										X (8:7-8:8)

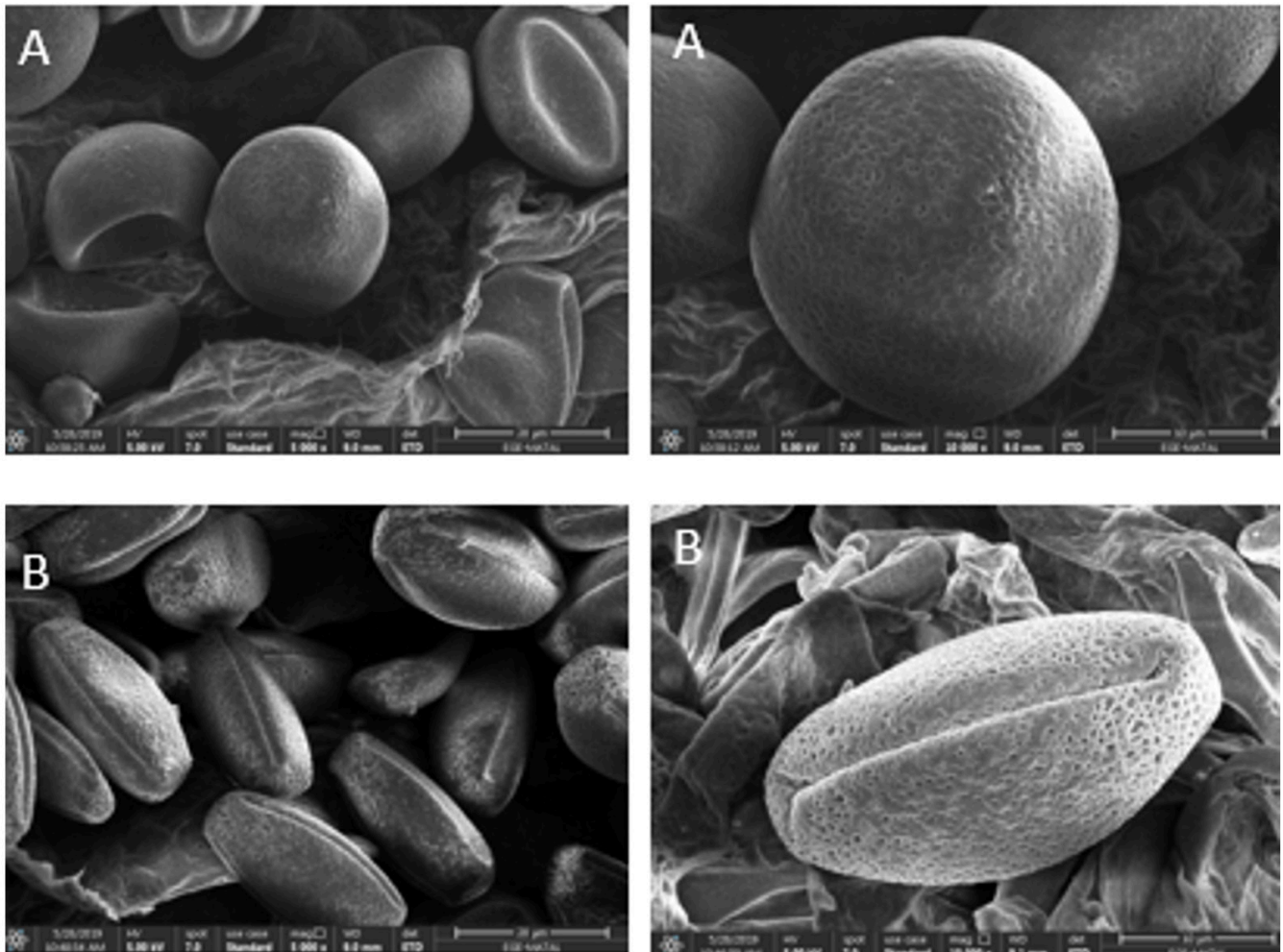


Figure 1. Scanning electron microscope image of pollen
 A: Inaperturate pollen in “Foça Karası” and “Mahrabası”.
 B: Tricolporate pollen in “Alphonse Lavallee”, “Cinsaut”, “Alicante Bouschet”, “Italia”, “Syrah”, “Cardinal”, “Yuvarlak Çekirdeksiz” and “Malbec”.

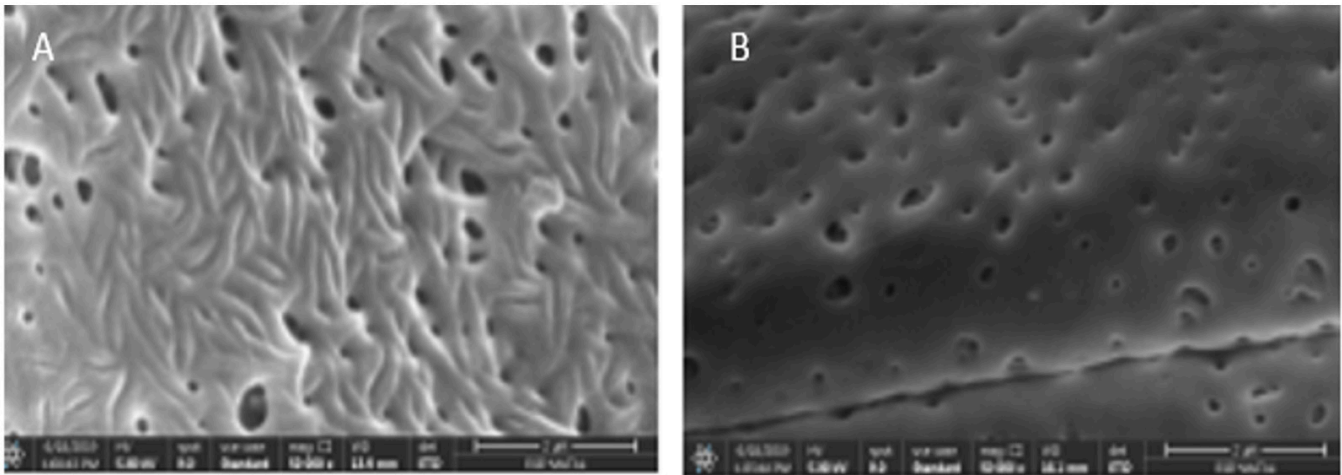


Figure 2. Pollen exine ornamentation image

A: Areolat pollen in “Foça Karası”, “Mahrabaşı”, “Alphonse Lavallee”, “Cinsaut”, “Malbec”, “Italia” and “Yuvarlak Çekirdeksiz”.
B: Without furrows pollen in “Alicante Bouschet”, Syrah” and “Cardinal”.

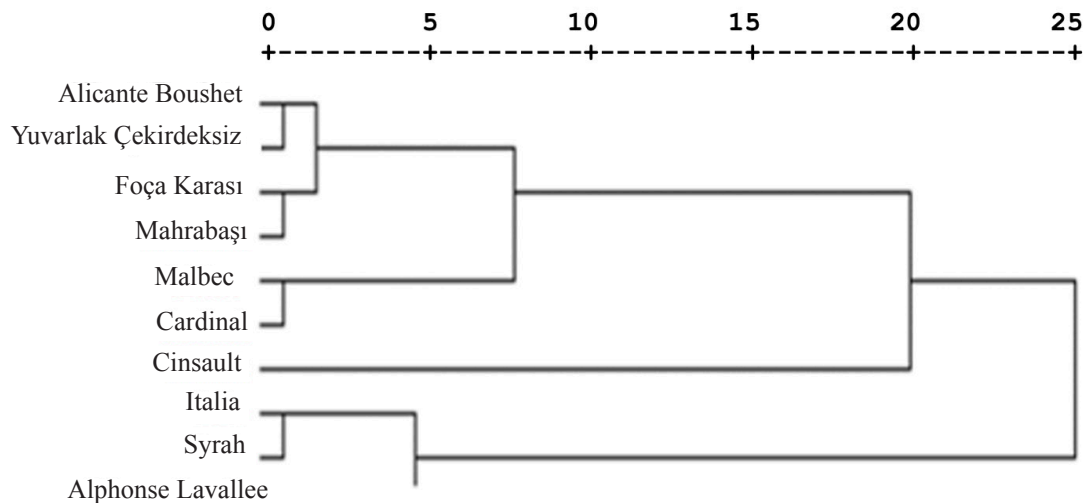


Figure 3. Dendrogram of hierarchical cluster analysis obtained by Ward’s clustering method

Pollen width sizes of the “Cardinal” studied has 13.96 μm , and length of pollen grains has 29.43 μm . In relation to the results reported by Marasalı et al. (2005) and Gökbayrak and Engin (2016), the “Cardinal” pollens were medium sized, the values obtained in our studies were higher than in both studies. The pollen width and length of “Yuvarlak Çekirdeksiz” were 19.09 μm and 26.27 μm , respectively. Roytchev et al. (1994) reported that pollen is same sized (the highest mean values - 24.13 and 24.04 μm) all investigated the seedless grape cultivars.

Vitis is characterized by its tricolporate pollens. However, there was difference in pollen shape in our study. Prolate-spheroidal pollen was found in the material from “Foça Karası”, whereas subsprolate pollen was “Mahrabaşı”. Perprolate were in “Alicante Bouschet”, “Syrah”, “Cardinal”, “Italia”, and prolate were in “Alphonse Lavallee”, “Malbec”, “Cinsaut”, “Yuvarlak Çekirdeksiz”. To confirm our findings, pollen shape and P/E

ratio change from one sample to another by Marasalı et al. (2005) and Gökbayrak and Engin (2016) for grapes. Roytchev (1997) reported in seedless cultivars, this ratio varies from 1.10 (cv. Seedless Red) to 2.08 (cv. Russalka), being < 2 for most of the cultivars. The elliptical oval shape of pollen grains is typical for most of the seedless grapes.

Reticulated pollen grains in members of *Vitaceae* were described by Erdtman (1952). The exine sculpturing of *Vitis* was reticulate, foveolate-perforate and that lumina size increased towards the poles reported by Faegri and Iversen (1989). Our study showed that in the ten grape cultivars, exine sculpturing was obscurely reticulate under SEM, and scrobiculate and striate at the mesocolpia and distinctly reticulate at and around the poles. An increase in lumina size towards the poles, observed by SEM, supports the results for Faegri and Iversen (1989). The results of this study showed that pollen can be used with SEM as a distinctive feature in

Vinifera cultivars.

As a result of this study, we found without furrows some of the examined cultivars (Cabello et al., 1994; Reille, 1966; Roytchev, 1997). These pollens were “Alicante Bouschet”, “Syrah”, and “Cardinal”, cultivars. Areolat is surrounded in exine were determined in cultivars of “Foça Karası”, “Mahrabası”, “Alphonse Lavallee”, “Cinsaut”, “Malbec”, “Italia” and “Yuvarlak Çekirdeksiz” (Figure 2).

The pollen viability was appreciated with TTC test, having verified that without furrows pollen grains presented similar percentages of viability such as, “Cardinal” (84.25 %), “Alicante Bouschet” (75.75 %) and “Syrah” (74.25 %) cultivars. Areolat pollen grain was viable, even though the lowest germination was recorded in “Malbec” (67.00 %), “Italia” (56.75 %), Yuvarlak Çekirdeksiz” (42.25 %), “Foça Karası” (26.50 %), “Alphonse Lavallee” (24.25 %), “Mahrabası” (20.25 %) and “Cinsaut” (11.75 %), respectively. However, the *in vitro* germination tests showed different results according to the pollen assayed. High pollen germination with agar in petri dishes was only observed in the “Mahrabası” (87.25 %, tricolporated form) (Table 5). Kelen and Demirtaş (2003) analysed in Burdur Dimriti, Sariemin, Tilki Kuyruğu, Razaki, Buzgülü, Siyah Buzgülü, and Siyah Gemre (*Vitis vinifera* L) table grape varieties pollen germination and pollen tube growth were only observed in the tricolporated form, as the acolporated pollen grains did not germinate. These results resemble previous data on *V. vinifera* from other authors that attributed nongermination to the lack of pores (Lombardo et al. 1978; Caporali et al. 2003; Lombardi 2007), on the other hand the low productivity might be owing to the presence of the acolporated pollen or to environmental conditions. Determination of pollen characteristics is extremely important, especially for grape varieties that will be subject to breeding studies. This work is of interest to the grape breeder for the purpose of avoid reduction in the productivity because the entity of grapevines producing acolporated pollen.

Pollen morphological features such as pore structure and ornamentation view are the most valuable variables for separating the grape species. The results of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering projection for species are quite common. The results from cluster analysis show that the examined members of the ten grape cultivars that fall into two main groups coincide with pollen morphological features. “Alicante Bouschet”, “Yuvarlak Çekirdeksiz”, “Foça Karası”, “Mahrabası”, “Malbec”, “Cardinal”, “Cinsaut” were included in the first group while “Italia”, “Syrah” and “Alphonse Lavallee”, were collected in the second group. First and second groups divided into different sub-groups (Figure 3).

Conclusion

Morphological characteristics of pollens showed significant differences among the ten grape cultivars.

Pollen width ranged from 10.12 µm to 22.44 µm, pollen length ranged from 16.26 µm to 29.91 µm, the length and width of pores are 112.40 µm to 397.45 µm and 97.06 µm to 245.68 µm, respectively.

According to the P/E ratio the ten cultivars are divided in

four groups: I - with perprolate (P/E > 2); II - with prolate (P/E 1.34-2.00); III - with subprolate (P/E 1.15-1.33); and IV – prolate-spheroidal (P/E 1.01-1.14). Especially “Mahrabası” (subprolate) and “Foça Karası” grape varieties (prolate-spheroidal) were separated from other varieties with their P/E feature.

High pollen germination with the agar in petri dishes was only observed in the “Mahrabası” (87.25 %, tricolporated form). To pollen grain viability with the TTC test, the highest germination was recorded in “Cardinal” (84.25 %), “Alicante Bouschet” (75.75 %) and “Syrah” (74.25 %) cultivars were without furrows pollen grains.

Some differences in size, polarity and ornamentation were observed among some of the studied cultivars in some cases among the ten grape cultivar. There were differences in pollen ornamentation in the cultivars studied. In this regard “Alicante Bouschet”, “Syrah” and “Cardinal”, cultivars were without furrows. On the other hand, areolat pollen determined in some cultivars were, such as “Foça Karası”, “Mahrabası”, “Yuvarlak Çekirdeksiz”, “Alphonse Lavallee”, “Cinsaut”, “Malbec” and “Italia”. Palynology of *Vitis vinifera* L., is an adequate and complementary observation for identification.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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