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APPLICATION OF CLUSTER ANALYSIS AND PRINCIPAL COMPONENT ANALYSIS IN PALYNOBIOMETRIC STUDIES OF SEEDLESS CULTIVARS AND HYBRID VINE FORMS (*VITIS VINIFERA* L.)

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Abstract

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The application of cluster analysis and principal component analysis in palynobiometric studies of 70 seedless cultivars and hybrid vine forms has been researched. It has been established that, depending on the studied cultivars' genetic similarity and remoteness, the most influential in their division into six larger groups are the most significantly varying indices from the pollen aperture complex - colp length, mesocolpium, colp width, equatorial axis and polar axis. There is a lack of discretion as regards the type of seedlessness, since cultivars with stenospermokarpic and stimulation parthenokarpy are positioned in one and the same group. The phenotype-palynological differentiation allows the use of the main aperture characteristics for the purposes of intraspecific taxonomy and ampelographic identification of seedless vine cultivars.

Key words: seedless vine cultivars, scanning electron microscopic observation, pollen grains, palynobiometry, cluster analysis, principal component analysis

Introduction

Contemporary research methods make it possible to perform an in-depth analysis of the micromorphological features of pollen grains in various vine cultivars. Palynological observations by means of a scanning electron microscope of seeded and seedless cultivars as well as of certain vine species have been conducted (Reille, 1966; Kasirszkaja and Kozma, 1978; 1981; Kasirszkaja, 1982, 1984; Linder and Linskens, 1978; Lombardo et al., 1976, 1978; Slimane and Askri, 1989; Roytchev et al., 1995;

Pandeliev and Roytchev, 1996; Inceoglu et al., 2000; Korkutal et al., 2004; Marasali, 2005). A number of classifications by different authors exist, according to the peculiarities of the microrelief of pollen grains (Wodehouse, 1935; Erdtman, 1952; Hyde and Adams, 1958; Amjad et al., 1969; Ahmedullah, 1983; Faegri, 1959; Faegri and Iversen, 1989; Roytchev, 1997). They are based on the external appearance and sizes of the most important pollen aperture complex elements - polar and equatorial axis, mesocolpium, apocolpium, colp and pore size. The fact that certain biometric correlations exist among

them, suggests a varying significance for the differentiation of cultivar remoteness. The application of cluster analysis and principal component analysis in studies of the correlation between the shape of surface formations and the sizes of pollen aperture complex elements will provide data on the degree of genotype – environment interaction and on the relative significance of individual palynobiometric indices, while also facilitate intraspecific taxonomy. The obtained results will scientifically justify the use of the main pollen aperture elements in taxonomy and ampelographic identification of seedless vine cultivars.

The objective of the present investigation is to determine the possibilities for application of cluster analysis and principal component analysis when dealing with results from palynobiometric studies of seedless cultivars and hybrid vine forms.

Materials and Methods

Within the period 1992-2005 a palynobiometric study of 70 seedless cultivars and hybrid vine forms was carried out. By means of a scanning electron microscope JEM 1200 with a scanning ASID 10 attachment, the most important pollen aperture complex elements – polar axis, equatorial axis, mesocolpium, apocolpium, length, width and depth of the colps, length and width of the pores (Terzijsky and Karageorgiev, 1989) were observed. The superficial microstructures of 100 pollen grains from each cultivar were measured directly from the screen, and at least 10 of each 100 grains were photographed. In the description of the pollen exine, the terminology adopted by Erdman (1952), Faergi (1959), Faergi and Iversen (1989), Sladkov (1962, 1967), Kuprijanova and Alashina (1967) was used. The division of the studied seedless cultivars and hybrid forms into homogeneous groups according to their genetic similarity and remoteness was performed through cluster analysis of the sizes of pollen aperture complex elements, and the division according to the relative significance of pollen aperture complex elements – through principal component analysis (PCA) (Ward, 1963; Duran and Odell, 1974; Everitt, 1979;

Philippeau, 1990). The collected data was processed through the statistical program SPSS. The forming of clusters (genotype groups) is presented graphically in a dendrogram, showing the order in which objects unite and the formation of separate groups.

Results and Discussion

The sizes of the most important pollen aperture complex elements of the studied 70 seedless cultivars and hybrid vine forms show that there are slight but specific differences among them in the absolute values of their micro-superficial structures (Table 1). As shown on the dendrogram, depending on the relative distance between them, they divide unevenly into six large groups consisting of a different number of cultivars (Figure 1). The first group includes 13 cultivars, divided into two subgroups; the second group includes 13 cultivars, the third - 5 cultivars, the fourth - 7 cultivars, the fifth - 15 cultivars divided into two subgroups, and the sixth group - 17 cultivars divided into two subgroups. The formation of a large number of groups and subgroups shows a higher degree of phenotypic differentiation in seedless cultivars as regards the pollen aperture complex indices. Each of the subgroups includes cultivars with a high degree of similarity in the studied indices, which therefore could be considered approximately identical. The relative distances between the limits of complete similarity are significant. The remotest, according to the absolute values of the studied indices, are the variants belonging to the first and sixth group. They include seedless cultivars with different genealogy, degree of parthenokarpy, areas of origin and distribution. According to the dendrogram data when palynological indices are used in cluster analysis there is a lack of discretion as regards the type of seedlessness, since cultivars with stenospermokarpic and stimulation parthenokarpy go to one and the same group (the first, second, fourth). It should be pointed out that the cultivar Trakyska Perla occupies a relatively independent position, while Sultanina Muskata divides conditionally, at an initial level of interpretation, the studied cultivars into two very large groups - the first con-

Table 1
Dimensions of the most important pollen aperture complex elements in the studied seedless cultivars and hybrid vine forms divided into groups, μm

№	Seedless cultivars and hybrid vine forms	Polar axis	Equatorial axis	Meso-colpium	Apocolpium	Colp length	Colp width	Colp depth	Pore length	Pore width
First group										
<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10</i>	<i>11</i>
37	Kishmish black	24.17	15.19	11.64	4.71	21.57	0.96	0.52	1.16	1.57
48	Kishmish Hyshrau	24.43	14.96	12.15	4.59	21.38	0.80	0.62	0.82	1.82
36	Early seedless	25.63	14.85	11.39	5.03	21.11	0.75	0.46	0.92	0.84
28	Korsa kishmish	23.73	15.54	12.59	4.76	20.30	0.75	0.61	0.62	1.61
65	Hybrid 3-6	24.41	13.62	10.85	5.34	21.50	1.05	0.59	2.22	1.14
66	Hybrid 17-2	24.96	14.43	10.15	5.61	22.57	0.87	0.52	2.00	1.18
64	Hybrid 20-4	26.10	13.84	10.88	5.97	21.81	0.73	0.50	2.03	1.24
56	Perlette	25.53	14.52	11.06	5.58	20.24	0.81	0.50	2.54	1.63
57	Sultanina gigas	24.78	14.59	10.96	5.31	20.17	0.96	0.61	2.84	1.32
55	Korint rose	24.87	19.91	11.56	5.84	19.91	0.88	0.53	2.53	1.42
29	Raucha white	23.73	15.60	12.59	4.53	20.26	0.87	0.50	0.96	1.54
52	Kishmish luchistii	23.01	14.25	10.90	4.59	19.12	0.84	0.48	3.06	1.68
70	Kondarev 6	26.44	16.46	11.06	5.81	21.08	0.83	0.60	2.65	2.29
Second group										
23	Gigant	22.76	15.07	11.09	4.47	20.55	1.00	0.63	1.37	1.56
30	Kishmish Irtishor	23.13	15.58	10.81	4.75	20.30	0.96	0.61	0.65	1.52
21	Slavjanka	23.30	15.16	10.82	4.93	21.37	0.95	0.60	0.86	1.47
45	Hybrid 21-17-41	22.91	15.26	10.61	4.50	21.16	0.84	0.55	2.19	1.83
26	Flame seedless	23.39	15.23	11.72	5.57	21.33	0.90	0.64	2.56	1.70
54	Ushaas nazely	22.77	16.25	11.67	5.12	19.89	1.16	0.64	2.46	1.38
24	Jangier	24.35	15.78	11.97	4.42	22.10	0.91	0.72	2.57	1.97
43	Beauty seedless	23.09	17.36	12.32	6.12	19.72	1.13	0.69	2.51	2.14
40	Korintsko black	21.21	17.79	13.13	5.21	19.29	0.91	0.76	2.13	2.00
62	Russalka 5 B	21.69	13.84	11.23	4.96	20.86	0.79	0.52	0.62	1.84
32	Hybrid 36-16	22.73	13.07	10.62	4.58	21.28	0.96	0.58	2.22	1.20
34	Nedelchev VI-4	23.97	12.59	9.49	4.80	20.60	0.72	0.44	2.17	1.02
67	Kishmish muscatni	23.76	11.74	10.43	4.64	23.03	0.94	0.60	1.05	1.02
Third group										
41	Kishmish VIRA	20.11	14.36	11.75	4.65	19.50	0.97	0.64	1.42	1.41
49	Early kishmish	20.72	14.21	11.41	4.88	18.40	0.79	0.47	0.70	1.73
44	Russalka 3	19.03	16.68	12.27	4.78	18.75	1.05	0.57	1.55	1.33
46	Kishmish Vatkana	19.94	15.75	12.06	4.84	18.27	0.94	0.60	2.06	1.94
3	Sultanina	21.78	14.97	10.70	5.27	17.90	1.63	0.94	2.62	1.51
Fourth group										
33	Russalka 1	20.18	11.85	9.45	4.12	17.34	0.67	0.53	2.31	1.61

Table 1 (continued)

1	2	3	4	5	6	7	8	9	10	11
51	Sermanly	20.98	12.85	10.22	3.96	18.33	0.77	0.48	2.31	1.58
35	Emerald seedless	21.29	11.82	9.66	4.07	18.74	0.70	0.51	0.87	1.87
25	Kishmish tjurkmenski	20.87	13.28	10.11	3.57	19.67	0.79	0.65	0.86	0.46
2	Korintsko white	19.47	12.53	8.94	3.75	16.43	1.54	0.94	2.21	1.37
1	Russalka	24.95	12.00	9.75	4.87	16.98	1.59	0.94	2.42	1.57
42	Trakyska perla	23.71	17.43	14.20	5.30	20.55	0.90	0.54	1.91	1.39
Fifth group										
31	Askery	24.56	17.21	11.94	5.04	22.37	0.84	0.46	0.52	1.34
50	Italia x Sultanina	24.57	17.09	12.21	5.68	21.49	0.94	0.52	0.91	1.62
53	Rody	25.16	17.40	13.65	6.27	22.05	1.04	0.67	1.38	1.77
39	Hybrid 23-4	24.44	17.70	12.79	3.80	21.90	0.84	0.57	1.83	1.50
58	Bljan	24.93	17.26	12.30	4.46	23.53	0.85	0.55	2.48	2.13
27	Seedless hybrid V-6	27.03	18.22	13.06	4.46	24.13	1.09	0.69	1.63	1.50
61	Tompson seedless	28.71	15.77	12.81	5.10	24.44	0.79	0.48	0.54	1.56
69	Apirena di Valettri	27.23	15.58	12.56	5.02	25.14	0.95	0.50	0.93	0.84
63	Hybrid 5-2	27.23	14.67	11.64	5.31	23.40	0.73	0.50	1.90	1.39
68	Apirena Bruni	27.22	15.18	11.37	4.88	23.28	1.13	0.59	2.28	1.36
38	Rusensko seedless	26.53	15.53	11.65	5.70	22.19	0.95	0.54	2.34	1.77
60	White seedless form	26.85	16.78	12.11	4.96	24.20	0.79	0.52	2.69	1.64
22	Kishmish moldavski	26.16	15.40	12.85	6.46	24.04	0.93	0.55	0.98	1.58
59	Russalka 2	29.77	17.07	12.23	4.25	26.10	0.98	0.62	3.34	1.43
47	Sultanina muscata	18.89	16.18	12.38	4.17	17.36	0.72	0.53	2.46	1.51
Sixth group										
9	Nishava	25.32	17.28	11.50	5.48	21.26	0.99	0.61	1.21	0.84
20	Vita	25.69	15.54	11.47	5.41	22.53	0.87	0.61	0.60	1.43
5	Kolarovets	24.82	17.74	12.56	4.74	23.04	1.00	0.80	1.57	0.90
11	Kondarev 10	24.51	15.85	11.61	4.61	19.90	0.98	0.62	2.79	1.98
12	Rushaki	24.64	15.32	11.65	4.42	21.17	0.90	0.64	2.15	1.45
6	Kara sultani	23.90	17.35	12.16	5.21	20.98	0.95	0.63	2.70	1.39
13	Russalka 5-A	23.74	15.34	11.40	4.75	21.03	1.00	0.81	0.52	0.62
15	Hybrid 720-19	23.30	14.38	11.05	4.50	21.23	0.93	0.62	0.62	0.52
16	Delight	24.36	14.33	11.44	5.38	22.24	0.91	0.62	2.67	1.49
17	Superior seedless	21.68	12.08	9.48	4.13	19.61	0.76	0.51	1.40	1.28
18	Ruby seedless	20.88	12.88	10.32	4.39	19.02	1.04	0.57	1.27	1.95
8	Focha seedless	21.80	14.64	11.38	5.28	19.77	1.04	0.71	2.25	1.45
14	Tarnau	22.15	14.03	11.77	4.40	19.00	0.92	0.83	2.23	0.61
19	Early superior seedless	21.47	13.70	11.01	4.44	19.30	0.82	0.58	0.70	0.50
4	Korint seedless	20.31	14.87	11.58	4.71	17.83	1.15	0.76	1.71	0.86
7	Nimrang x Sultanina	21.39	16.26	11.86	4.12	19.71	0.98	0.67	0.82	0.49
10	Red seedless	19.41	17.69	12.69	4.50	18.52	1.11	0.61	1.17	0.75

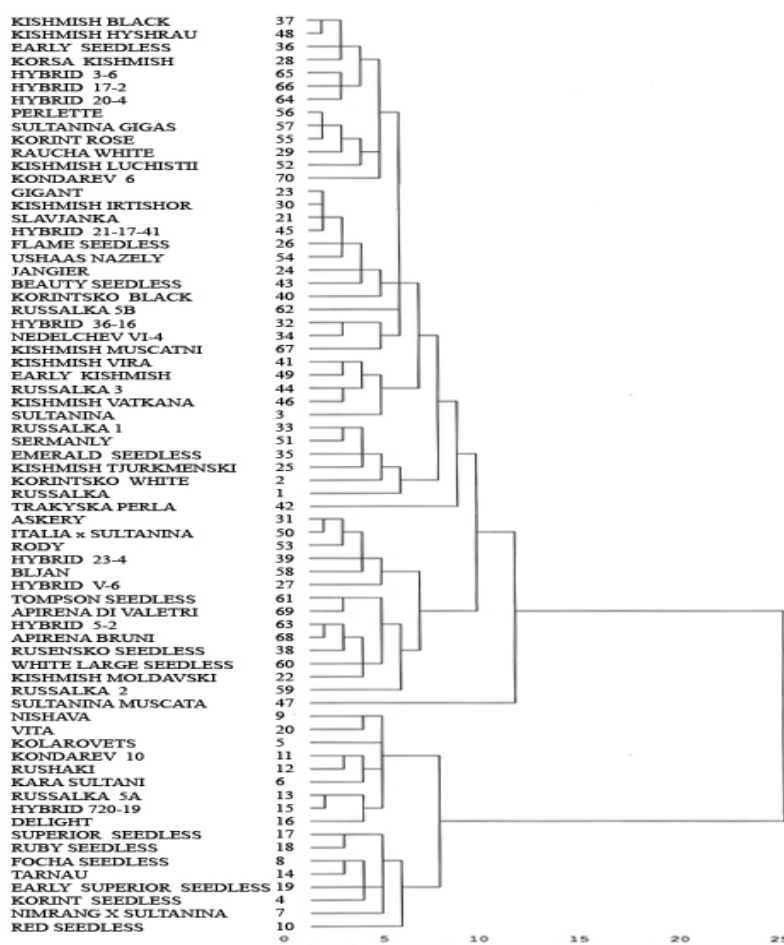


Fig. 1. Dendrogram-clustering of seedless vine cultivars according to the pollen aperture complex elements - polar axis, equatorial axis, mesocolpium, apocolpium, length - width - depth of the colp, length and width of the pore

sisting of 53 cultivars and the second - of 17 cultivars.

The results from the principal component analysis show that out of the nine possible components only four explain 75.144% of the total variation (Table 2). The first principal component explains 26.796% of the total variation in the studied seedless cultivars. The indices colp length, mesocolpium, colp width, equatorial axis and polar axis exert the strongest influence on the division of cultivars into groups, since they are characterized by the highest relative variation rate.

The indices colp width, equatorial axis, colp length and mesocolpium are the most significant in the second principal component. It explains 21.8969% of the total variation. The differences between the first

and the second principal component in the degree of explanation of the total variation are minor. The most significant indices in these two components are almost identical - colp length, colp width, mesocolpium and equatorial axis. That is why it could be stated that these indices explain to the greatest extent 48.665% of the total variation in the current investigation.

The third principal component explains 14.021% of the total variation, mainly through the indices pore width and pore length, and the fourth principal component explains 12.458% of the total variation, mainly through pore width, pore length and colp depth.

The pollen grains of all studied cultivars and hybrid forms are tricolporate. The most significantly vary-

Table 2
Results from the principal component analysis of the seedless cultivars and hybrid forms

Indices	Principal components			
	1	2	3	4
Polar axis	0.571	0.079	-0.400	0.369
Equatorial axis	0.589	0.680	0.151	-0.047
Mesocolpium	0.672	0.637	0.050	-0.194
Apocolpium	0.497	0.282	-0.107	0.017
Colp length	0.734	-0.638	0.017	0.041
Colp width	-0.643	0.704	-0.121	-0.007
Colp depth	-0.228	0.311	0.424	0.552
Pore length	0.155	-0.102	0.600	0.561
Pore width	0.100	-0.067	0.715	-0.569
Explained % of the total variation	26.796	21.869	14.021	12.458

ing indices from the pollen aperture complex for the cultivars from the first group possess comparatively close absolute values, which are as follows: for the polar axis - from 23.01 μm (Kishmish luchistii) to 26.44 μm (Kondarev 6); for the equatorial axis - from 13.62 μm (Hybrid 3-6) to 19.91 μm (Korint rose); for the mesocolpium - from 10.15 μm (Hybrid 17-2) to 12.59 μm (Korsa kishmish and Raucha white); for the apocolpium - from 4.53 μm (Raucha white) to 5.97 μm (Hybrid 20-4) and for colp length - from 19.12 (Kishmish luchistii) to 22.57 μm (Hybrid 17-2) (Table 1). The differences between the parameters of the remaining studied indices vary very slightly. The pollen grains have an elliptical shape. The colps are long, with even contours, almost equally deep, opened along their entire length and with prominent pores. The exine surface in the equatorial part is characterized by a low relief, covered with shallow coves and holes, predominantly with a rounded periphery. Apocolpia are protruding, with a slightly undulating surface cut by coves with varying depth and individual projecting points (Figures 2 A,B,C).

The differences between the sizes of the studied pollen aperture elements for the cultivars from the second group are insignificant, too: for the polar axis - from 21.21 μm (Korintsko black) to 24.35 μm (Jangier); for the equatorial axis - from 11.74 μm

(Kishmish muscatni) to 17.79 μm (Korintsko black); for the mesocolpium in the previous two cultivars - from 10.43 μm to 13.13 μm ; for the apocolpium - from 4.42 μm (Jangier) to 6.12 μm (Beauty seedless) and for colp length - from 19.29 μm (Korintsko black) to 23.03 μm (Kishmish muscatni). The pollen grains have an oval shape. The colps are wide, relatively deeply opened without a demarcating ridge, with unclear contours and a microverrucose bottom. The mesocolpium ultrastructure is perforated and pitted with uneven, plateau-like areas. The apocolpia are domed, covered with dispersed coves and pits, predominantly having a round shape (Figures 2 D,E,F).

The cultivars from the third group differ from the others, having comparatively the smallest sizes of the measured indices: for the polar axis - from 19.03 μm (Russalka 3) to 21.78 μm (Sultanina); for the equatorial axis - from 14.21 μm (Early kishmish) to 16.68 μm (Russalka 3); for the mesocolpium - from 10.70 μm (Sultanina) to 12.27 μm (Russalka 3); for the apocolpium - from 4.65 μm (Kishmish VIRA) to 5.27 μm (Sultanina) and for colp length - from 17.90 μm (Sultanina) to 19.0 μm (Kishmish VIRA). The pollen grains have an elongated spherical shape. The colps are of medium length, shallowly opened, wide, with uneven contours, a microverrucose bottom and prominent pores. The exine surface in the mesocolpium area

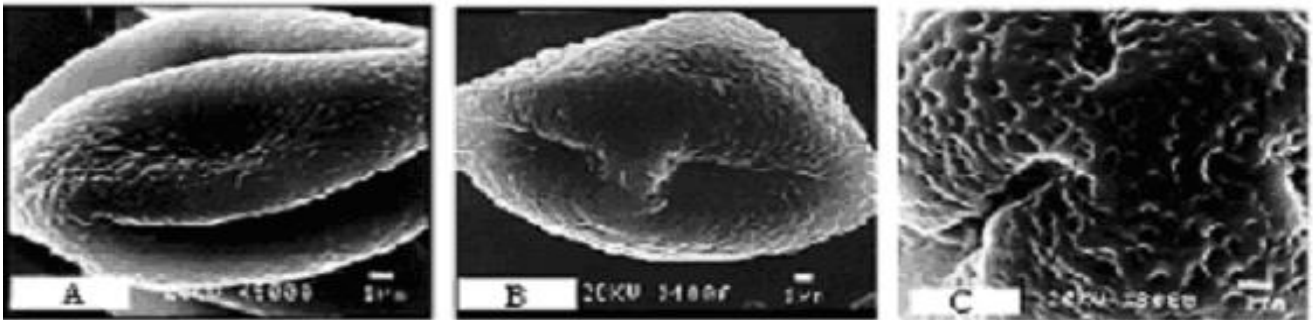


Fig. 2. A,B,C. Submicroscopic structure of the pollen exine and the aperture complex elements in the cultivars Perlette, Korint rose, Kishmish luchistii - first group

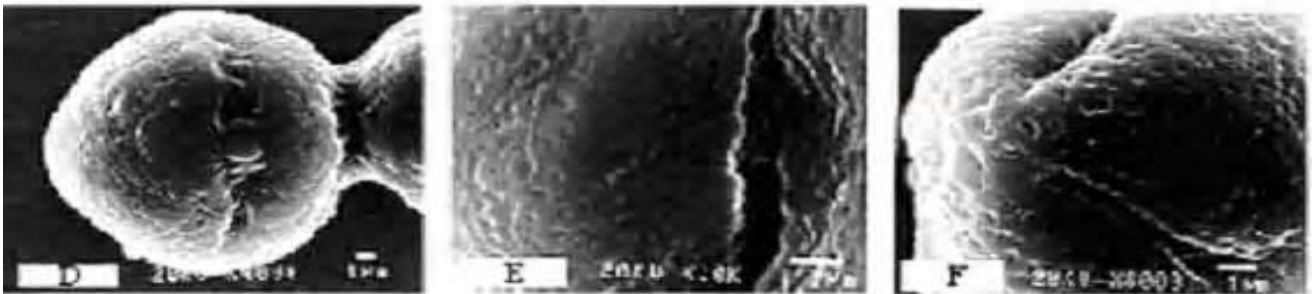


Fig. 2. D,E,F. Ultrastructure of the pollen exine in the apocolpium and colps area in the cultivars Nedelchev VI-4, Hybrid 21-17-41, korintsko black - second group



Fig. 2. G,H,I. General view of the ultrastructural organization of the pollen exine in the cultivars Sultanina, Kishmish Vatkana, Kishmish VIRA - third group



Fig. 2. J,K,L. Microrelief of the pollen exine - mesocolpium, colps, pores and apocolpium in the cultivars Kishmish tjurkmenski, Russalka, Russalka 1 - fourth group

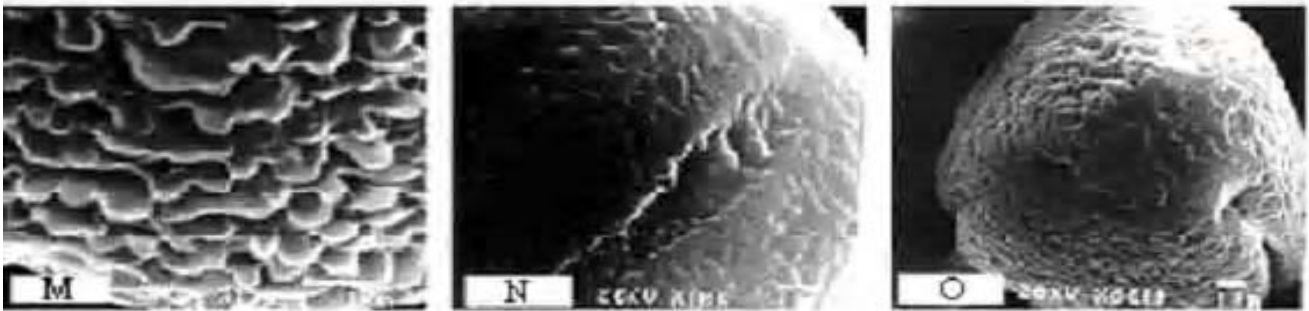


Fig. 2. M,N,O. Submicroscopic aspect of the pollen exine in the area of the mesocolpium, apocolpium and bottom of the colps in the cultivars Tompson seedless, Askery, Sultanina muscata - fifth group

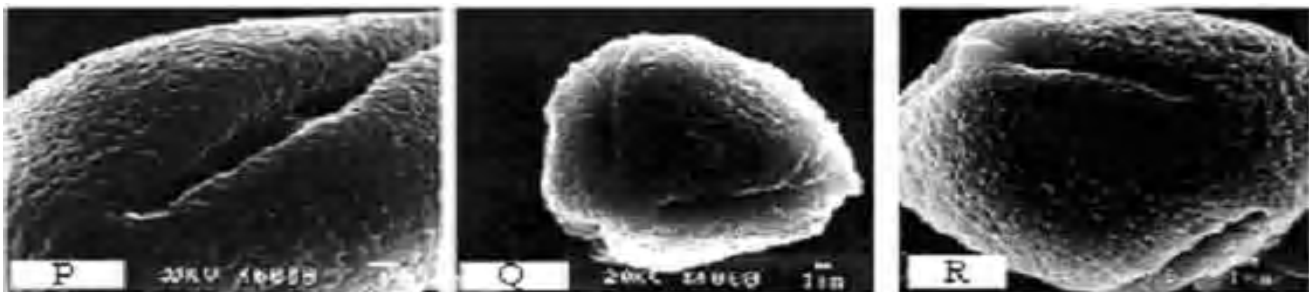


Fig. 2. P,Q,R. Ultrasculpture of the pollen exine - colps, mesocolpium and apocolpium in the cultivars Delight, Hybrid 720-19, Tarnau - sixth group

possesses a clear undulating structure and is often divided by perforations varying in shape and size (Figures 2 G,H,I).

For the cultivars from the fourth group the studied indices have comparatively the highest values in Russalka: polar axis – 24.95 μm ; Trakyska perla: equatorial axis – 17.43 μm ; mesocolpium – 14.20 μm ; apocolpium – 5.30 μm and colp length – 20.55 μm . The lowest values of the tested indices were registered mainly in Korintsko white: polar axis – 19.47 μm , mesocolpium – 8.94 μm , colp length – 16.43 μm ; Emerald seedless: equatorial axis – 11.82 μm ; Kishmish tjurkmenski: apocolpium – 3.57 μm . The pollen grains have an elongated elliptical shape. The colps are long, ending near the polar part, deeply opened with uneven bottom and clearly outlined equatorial pores. The mesocolpiums are perforated reticulately, characterized by low irregular undulations in separate areas, split under frontal scanning by perforations and pits with various shape and sizes. In the apocolpium ultrastructure chaotically oriented uneven

folds and coves predominate (Figures 2 J,K,L).

The cultivars from the fifth group are characterized by the highest average values of the registered indices. The data on the polar axis shows that it varies from 18.89 μm (*Sultanina muscata*) to 29.77 μm (Russalka 2), while the colp length varies from 17.36 μm to 26.10 μm in the same cultivars. It should be pointed out that as regards the first index, its values for the remaining cultivars are not lower than 24.44 μm (Hybrid 23-4), and for the second index - not lower than 21.49 μm (Italia x Sultanina). The sizes of the equatorial axis vary from 14.67 μm (Hybrid 5-2) to 18.22 μm (Seedless Hybrid V-6); the mesocolpium sizes - from 11.37 μm (Apirena Bruni) to 13.65 μm (Rody); the apocolpium sizes - from 3.80 μm (Hybrid 23-4) to 6.46 μm (Kishmish moldavski). The pollen grains have an oval-elliptical shape. The colps are long, extended in the equatorial part and narrower towards the polar top, shallowly opened, with a verrucose uneven bottom. The ultrastructure in the mesocolpium area is perforated reticulately with holes,

dimples, deep folds with irregular shape and individual projecting points. The apocolpiums are slightly protruding, with undulating surface and clearly visible, when magnified 20 000 times, smooth and thick rope-like domed protuberances, which often intertwine forming branches and deep pits (Figures 2 M,N,O).

The biometric data on the cultivars from the sixth group presents the same diversity in the values of the tested indices. The largest polar axis belongs to the cultivar Vita – 25.69 μm , and the smallest polar axis - to Red seedless – 19.41 μm ; for the equatorial axis the corresponding cultivars are Superior seedless – 12.08 μm and Kolarovets – 17.74 μm ; for the mesocolpium - Superior seedless – 9.48 μm and Red seedless – 12.69 μm ; for the apocolpium - Korint seedless – 17.83 μm and Kolarovets – 23.04 μm . The pollen grains have an oval-elliptical shape. The colps are long, slightly extended in the equatorial part, with uneven bottom and deeply opened. The exine surface in the mesocolpium area has a low relief and it is slightly folded in certain parts, with a large number of holes and coves with different figure contours. The apocolpiums possess a prominent plateau-like part; they are slightly bulging, cut by coves, streaks and perforations (Figures 2 P,Q,R).

The observed microstructural peculiarities of the pollen exine among the cultivars and hybrid forms from the subgroups of the first, fifth and sixth group, are within the limits of their ampelographic character. The investigations carried out through a scanning electron microscope as well as the statistical processing of the obtained results show that the pollen aperture complex indices in seedless cultivars can be used for the purposes of ampelographic and taxonomic studies.

Conclusions

There are no significant differences in the shape of the superficial pollen microstructures – polar axis, equatorial axis, mesocolpium, apocolpium, length-width-depth of the colps and pores in the studied 70 seedless cultivars and hybrid vine forms. However, each of them is characterized by specific features of the exine microrelief and the sizes of aperture com-

plex elements. Depending on the studied cultivars' genetic similarity and remoteness, the most influential in their distribution in six larger groups are the most considerably varying indices from the pollen aperture complex - colp length, mesocolpium, colp width, equatorial axis and polar axis. Four of the principal components explain 75.144% of their total variation. When palynological indices are used in cluster analysis and principal component analysis, there is a lack of discretion as regards the type of seedlessness, since cultivars with stenospermokarpic and stimulation parthenokarpy are situated in one and the same group. In the formed groups and subgroups of cultivars and hybrid forms, according to the relative significance of separate indices, a considerable similarity and resemblance in the ultrasculptural ornamentation of the pollen exine are observed despite their individual identity. The established phenotype-palynological differentiation allows the use of the main aperture characteristics for the purposes of intraspecific taxonomy and ampelographic identification of seedless vine cultivars.

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