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Palynobiometric studies in vines (*Vitis vinifera* L.)

by

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S u m m a r y : A palynobiometric investigation of the most important aperture elements of the exine complex in seedless grape cvs Russalka, Korintsko White, Sultanina and the seeded cv. Bolgar was performed by using scanning electron microscopy technique. After direct measurements from the screen, it was established by a scanning image device that a 40-60 pollen grain sample is representative for the polar and equatorial axes, mesocolpium, colp length and apocolpium, while 30-50 pollen grains are representative for the colp width and colp depth.

K e y w o r d s : palynobiometry, electron microscopy, pollen, representative sample.

Introduction

The number of palynological studies in vines is scarce in comparison with the large number of cultivars. In the description of the pollen exine microrelief, the separate elements - polar axis, equatorial axis, mesocolpium, apocolpium, length, width and depth of the colp are biometrically characterized by measuring. According to KURSANOV (1978) and SILAEVA and SILAEV (1979), in electron microscopic investigations, the qualitative analysis of vegetative objects, which is rather subjective, occupies a predominant position. Therefore, the determination of quantitative relations among the different indices contributes to the development of ultrastructure morphophysiology.

Of the scanning electron microscopy investigations of grapevine pollen, known so far, no indications were made for the number of pollen grains subjected to measurements nor the rate of significance for the data obtained (LINDER and LINSKENS 1978; TOMPANÉ KASIRSZKAJA and KOZMA 1981; TOMPANÉ KASIRSZKAJA 1982, 1984, 1985; AHMEDULLAH 1983; BEN SLIMANE and ASKRI 1989). Measurements are most often performed, using a limited number of microphotographs, which sharply decreases the sample size, the exposition diversity, and which exerts a negative effect on the precision of the estimated results. No special palynobiometric investigations of the aperture complex elements in vines has been proposed in literature so far. The objective of the present study was to establish the optimum mean value for scanning electron microscopy investigation with regard to the number, shape and dimensions of pollen grain apertures in grapevine cultivars.

Material and methods

The investigations were carried out with pollen from seedless grape cvs Russalka, Korintsko White, Sultanina and the seeded cv. Bolgar (Regina), which was collected at the time of flowering in 1992-1994 from clusters located on different nodes along the shoot. The dimensions of the aperture complex elements were taken directly from the screen of the scanning image device on JEM 1200 EX electron microscopy (magnification 3000 x) upon 100 normally shaped pollen grains from each cultivar tested. The independent comparative study on the values of all important indices, estimated from micrographs, photofilms and directly from the screen, have shown that no significant differences existed in the accuracy of measurements. The direct measuring of aperture complex elements at the pollen grain scanning enables the observation of an unlimited number of objects at different exposition and magnification.

To establish the least sample size at which significant results are obtained, 7 samples including different numbers of pollen grains (20, 30, 40, 50, 60, 80 and 100) were taken at random from each cultivar. The number of pollen grains (replications) was calculated by the formula of URBAH (1964). The statistical treatment of experimental data was made by the methods described by LAKIN (1990).

Results and discussion

Tab. 1 shows the most important indices involved in the biometrical characteristics of grapevine pollen. It can be seen that the arithmetic means for all characters change significantly depending on the specific sample size. With the four cultivars, the differences in the arithmetic means for all characters are in the range of 0.5 - 3.0 µm. The values for the different characters' dimensions of the tested cultivars change in a small interval of 0.2 - 2.5 µm. By these indices, the differences in the biometrical characteristics of pollen between seeded and seedless grape cultivars are not significant.

The comparatively high coefficient of variation determines the differences established in the values of characters, involved in the scheme of the experimental work. The increase of the sample size was related to a decrease of the coefficient of variation in the tested indices for all cultivars. For the polar axis, equatorial axis, mesocolpium, colp length and apocolpium, the coefficient of variation shows

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Table 1

Change of arithmetic means as depending on the size of the sample (μm)

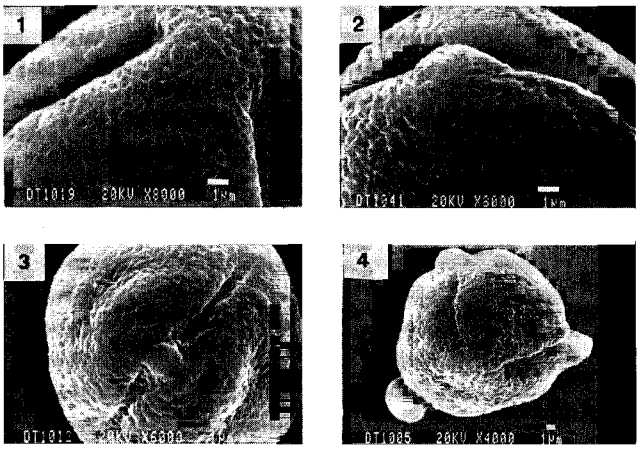
CULTIVAR	20	30	40	50	60	80	100
Polar axes							
1. Russalka	23.03	21.03	24.83	22.49	22.95	22.30	22.21
2. Korintsko white	18.35	19.40	19.26	19.81	19.50	19.39	19.33
3. Sultanina	23.72	23.36	21.63	21.00	21.09	21.76	21.99
4. Bolgar	21.38	21.40	20.35	22.21	20.38	20.83	21.21
Equatorial axis							
1. Russalka	14.78	13.83	12.59	12.55	12.61	12.61	12.99
2. Korintsko white	12.77	12.41	12.74	12.52	12.59	12.58	12.55
3. Sultanina	15.03	14.35	14.91	14.96	14.97	14.78	14.72
4. Bolgar	14.04	12.92	12.87	12.68	12.89	13.13	12.81
Mesocolpium							
1. Russalka	9.32	9.84	10.15	9.64	9.74	10.01	10.08
2. Korintsko white	8.93	8.69	8.83	8.91	8.94	8.89	8.82
3. Sultanina	10.91	10.55	10.68	10.67	10.72	10.70	10.68
4. Bolgar	8.77	8.75	8.81	9.21	9.28	8.99	9.08
Apocolpium							
1. Russalka	4.86	4.71	4.78	4.72	4.66	4.79	4.74
2. Korintsko white	3.87	3.95	3.98	3.65	3.74	3.77	3.80
3. Sultanina	5.45	5.08	5.48	5.58	5.24	5.40	5.39
4. Bolgar	4.51	4.71	4.08	4.15	4.53	4.35	4.37
Length of the colp							
1. Russalka	17.27	18.05	16.61	17.27	17.60	17.57	17.49
2. Korintsko white	17.59	16.85	16.37	17.04	16.73	17.12	16.69
3. Sultanina	16.51	18.18	19.45	19.41	19.58	18.27	18.48
4. Bolgar	18.18	19.11	17.65	17.71	18.48	18.14	18.16
Width of the colp							
1. Russalka	1.40	1.08	1.70	1.82	1.20	1.32	1.52
2. Korintsko white	1.74	1.60	1.69	1.67	1.66	1.65	1.67
3. Sultanina	1.89	1.53	1.54	1.67	1.66	1.55	1.62
4. Bolgar	2.03	1.85	1.93	1.94	1.92	1.93	1.94
Depth of the colp							
1. Russalka	0.90	0.84	1.32	1.29	1.02	1.11	1.07
2. Korintsko white	1.37	1.36	1.23	1.26	1.26	1.30	1.31
3. Sultanina	1.57	1.40	1.36	1.34	1.32	1.28	1.33
4. Bolgar	1.35	1.27	1.36	1.24	1.36	1.33	1.35

Table 2

Values of most important biometrical indices (μm)

INDICES	CULTIVARS			
	Russalka	Korintsko white	Sultanina	Bolgar
Polar axis				
1. Arithmetic mean, $\bar{x}$	22.21	19.33	22.00	21.21
2. Coefficient of variation, S%	20.93	11.15	17.45	10.02
3. Index of precision, S$\bar{x}$%	2.10	1.12	1.75	1.00
Equatorial axis				
1. Arithmetic mean, $\bar{x}$	13.00	12.55	14.72	12.81
2. Coefficient of variation, S%	21.34	13.97	11.78	12.22
3. Index of precision, S$\bar{x}$%	2.14	1.40	1.18	1.22
Mesocolpium				
1. Arithmetic mean, $\bar{x}$	10.08	8.82	10.68	9.08
2. Coefficient of variation, S%	18.49	14.74	11.11	14.21
3. Index of precision, S$\bar{x}$%	1.85	1.48	1.11	1.42
Apocolpium				
1. Arithmetic mean, $\bar{x}$	4.74	3.80	5.39	4.37
2. Coefficient of variation, S%	21.90	24.83	21.34	20.13
3. Index of precision, S$\bar{x}$%	2.20	2.49	2.14	2.02
Length of the colp				
1. Arithmetic mean, $\bar{x}$	17.49	16.69	18.48	18.16
2. Coefficient of variation, S%	18.28	13.14	11.49	18.71
3. Index of precision, S$\bar{x}$%	1.80	1.32	1.15	0.87
Width of the colp				
1. Arithmetic mean, $\bar{x}$	1.52	1.67	1.62	1.94
2. Coefficient of variation, S%	41.72	27.76	31.77	20.93
3. Index of precision, S$\bar{x}$%	4.14	2.79	3.19	2.10
Depth of the colp				
1. Arithmetic mean, $\bar{x}$	1.07	1.31	1.33	1.35
2. Coefficient of variation, S%	37.70	38.66	29.70	26.76
3. Index of precision, S$\bar{x}$%	3.79	3.88	2.89	2.68

that the investigation has been carried out with satisfactory accuracy ($p \leq 3$). For this group of aperture elements, the comparatively high coefficient of variation for the apocolpium data should be noted which confirms the average variability of this character, established earlier. (ROYTCHEV *et al.* 1994). These data show that for the first 5 characters, the sample of 40-60 pollen grains could be taken as representative. The highest variation is observed in colp width and depth, in most cases and in a sample size of 80-100 pollen grains, the experimental accuracy exceeding 3. This dependency has also been established from the data in Tab. 2 for the pointed two characters of most cultivars. At this state of the experimental work, the sample size which could guarantee the obtaining of significant information even at 5 % accuracy, is very large. For the colp width it is in the range of 48.67 for cv. Bolgar to 193.39 in cv. Russalka, and for colp depth from 14.66 in cv. Sultanina to 166.06 in cv. Korintsko White. For these two indices, the varietal differences are very great. The significant fluctuations in the necessary sample size are due to the high coefficient of variation. Therefore, a special additional investigation was carried out on the changes of the colp width and depth as depending on the sample size. These two indices were measured at different pollen grain expositions. It turned out that the highest precision and the least variations in the colp width and depth dimensions were obtained when pollen grains were in the positions shown in Figs. 1-4. The coefficient of variation for the colp width reached 11.73 %, and that for colp depth –17.8 %.



Figs. 1-4: Exposition of pollen grains for the most precise measuring of colp depth in cvs Russalka (**1**), Korintsko White (**2**), Bolgar (**3**) and Sultanina (**4**).

The experimental data, obtained from the additional investigation, allow the conclusion that with the methods, already described, the necessary sample volume for the colp width and depth should be 30-50 pollen grains (Tab. 3). The index of precision drops to ≤ 2 %, which guarantees that the information obtained is representative. For the determination of the sample size, no exact limits are fixed, as measurements of all indices at a single pollen grain exposition are just impossible.

On the basis of the scanning electron microscopic and micrometric measuring of pollen grains from several grapevine cultivars, the following conclusions could be drawn:

1. The direct measuring of the pollen aperture complex elements from the screen of the scanning image device allows to comprise the whole exposition diversity of the observed object. It improves the precision and effectiveness of the experimental work.

2. Significant information for the values of the polar and equatorial axes, the mesocolpium, the colp length and the apocolpium could be obtained by a mean sample size of 40-60 pollen grains.

3. The precision of the experimental work at the investigation of the pollen aperture complex indices colp width and depth could be increased to 98 % if the width is measured at front exposition with clearly outlined colp ends, and the depth at a very good vision of the bottom and tip of one of the vertical colp walls (sides). By doing so the sample size of 30-60 pollen grains is representative for these indices.

Table 3

Size of pollen grain samples; number of grains at $p < 5$

CULTIVAR	Polar axis	Equatorial axis	Mesocolpium	Apocolpium	Length of the colp	Width of the colp	Depth of the colp
Russlka	48.67	50.55	37.98	53.29	37.12	46.69	35.20
Korintsko white	31.08	21.68	24.14	68.50	43.16	19.36	24.46
Sultanina	33.83	16.59	30.85	50.59	34.66	24.66	18.20
Rolgar	25.10	16.59	22.43	45.02	33.00	27.38	30.76

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