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CYTOGENETIC RESEARCH INTO SEEDLESS AND NATIVE BULGARIAN SEEDED VINE CULTIVARS (*VITIS VINIFERA* L.)

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Abstract

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A cytogenetic investigation of 29 seedless and 70 native Bulgarian seeded vine cultivars has been conducted. It has been found that only one of the seedless cultivars is polyploid with a doubled chromosome number (2n=76), while all the rest are diploid. Triploidy (2n=57) is not one of the possible causes for the appearance of parthenocarpy in vine, and the discovered triploid seedlings originate from valent crosses between tetraploid (4n) and diploid (2n) cultivars. The cluster in triploid forms is comprised almost entirely of seedless berries, and the larger-sized seeded berries are extremely rare. The native vine cultivars from Bulgaria do not include polyploid forms. Only a single cytochimera has been observed, while all the others are diploid – 2n=38. The scanning electron microscope examination of pollen grains indirectly confirms the diploid chromosome number in the studied seedless and seeded vine cultivars.

Key words: cytogenetic analysis, triploidy, seedless and native vine cultivars, pollen, scanning electron microscopy research (SEM)

Introduction

The cytogenetic method has not been widely applied for researching into the causes for parthenocarpy in vine. These studies in different vine cultivars are limited due to the large diploid number of chromosomes – 2n=38, which are situated very close to one another in the metaphase lamellae and have small sizes of 0.4 – 0.8 μm. Methodological difficulties in the preparation of the material for karyological investigations are still encountered nowadays, but the determination of morphology and chromosome number is important for the selection-genetic studies of each cultivar. Topale (2011) investigated the polyploidy of 826 cultivars, 820 of which are diploid, and he considers that, despite the existing relativity, it is the most accurate to determine the chromosome number in vine using the meristem from tips of shoots and adventive roots. There are few cytogenetic investigations of seedless vine cultivars and idiograms of their karyotype are not available (Ghimpu, 1941; Randhawa and Lyer, 1960; Sudharsan and Seethaiah, 1973; Lavie 1970; Haas et al., 2000). Apart from sex hybridization between diploid vine cultivars and inbreeding (Todorov, 2009), it is possible to use another method for the development of seedless forms – through triploidy (Olmo, 1937; Scherz, 1940; Mitsukuri and Hayashi, 1953; Patel and Olmo, 1957; Wakana et al., 2007). This selection direction presumes the presence of tetraploid vine forms, having originated spontaneously in the cultivated plantations, or created on purpose by crossing with other diploid cultivars. The hybrid seeded progeny consists of diploid (2n), triploid (3n) and tetraploid (4n) seedlings. Only after direct counting of chromosomes, it is possible to separate the triploid hybrid plants and ampelographic studies can be carried out in order to establish the commercial efficiency of triploidy as a different method for the production of seedless forms. In Bulgaria a diverse native vine assortment is available, created as a result of many years of selection, which should be the subject of cytogenetic investigations. The chromosome number of some of the most widely distributed seeded and seedless cultivars has been reported by Genchev and Zankov (1964); Dimitrov and Gadeva (1974); Angelov (1987); Tsolova (1990). The pur-

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pose of the current research is to determine the chromosome number of well-known seedless and native Bulgarian seeded vine cultivars.

Materials and Methods

Karyological studies have been conducted using cultivars and clones from the ampelographic collections of the National Institute of Viticulture and Wine Making "Magarach" – Yalta, the National Institute of Viticulture and Wine Making in Kishinev – Republic of Moldova, and some selection forms from Bulgaria. The somatic chromosome number of 10 collection plants from each cultivar has been determined, this being one of the most significant karyological characteristics, which makes it possible to analyze their influence on the phenotypic manifestation of the different morphological traits, the causes for pollen sterility in triploids, etc. At the beginning of the investigations the chromosome number was determined in tips of young roots from rooted cuttings, included in permanent preparations obtained in accordance with the standard methods in cytology (Raibin, 1967). The fixation was conducted in different solutions of Navashin, Levitski, Karnua, and after that the materials were washed in running water and immersed into ethyl alcohol solutions with rising concentration. Their following treatments include the preparation of microtome cuts with thickness of 8-10 μm, attaching to slides, removing paraffin and staining with ferrum-hematoxylin according to Heidenhain**.** In order to study a large number of hybrid plants in a short time, a faster way for the development of preparations was used – a propionate-orcein or propionate-lacmoid method for chromosome staining (Topale, 1983). A scanning electron microscope observation of the micro-morphological specific features of pollen grains in the various seedless and seeded vine cultivars was performed (Roychev, 2008).

Results and Discussion

The analysis of the cytological investigations of 29 seedless vine cultivars grown in different parts of the world, shows that the majority of them are diploid with a somatic chromosome number 2n=38, and only the clone with large-sized berries Kishmish belai krupnoiagodnai is a spontaneous tetraploid with 2n=76 (Figure 1). 22 of them have become a subject of cytological research for the first time. The data on the cultivars Askery, Kishmish belai ovalnai, Kishmish belai krupnoiagodnai, Kishmish mramornai, Kishmish rozovai, Korinka belaia and Korinka chernaia, entirely supports the results obtained by other authors (Table 1). The information about the ploidity of Kishmish belai krupnoiagodnai completely corresponds to the cytological results for the cultivar with large-sized berries Sultanina gigas, reported by Olmo (1935), which prove for the first time that this cultivar is a naturally generated polyploid. Studies confirm that, in accordance with the law of homologous series, these are analogous somatic alterations, which appeared in both seedless cultivars

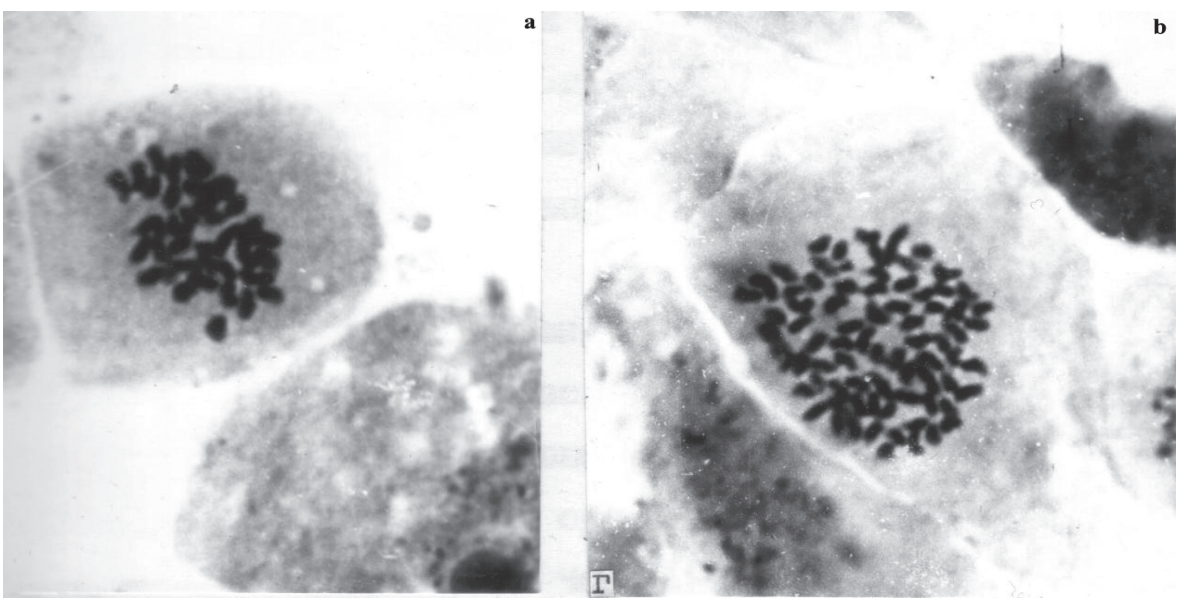

Fig. 1. Metaphase lamellae with chromosomes:
a - Kishmish belai ovalnai – 2n=38; b - Kishmish belai krupnoiagodnai – 2n=76, x 2660

as a result of a genomic mutation, in spite of the differences in the years and geographical locations of their cultivation (Vavilov, 1987).

The clone Kishmish belai krupnoiagodnai is a large-berry somatic (bud) mutation of the diploid cultivar Kishmish belai ovalnai, which radically differs from the initial cultivar in a number of agrobiological and technological characteristics (Iakimov and Kovshova, 1968). The cluster has a cylindrical shape, with one or two wings, and often with fewer berries in the middle section (Figure 2). The average cluster weight is 400-687.5 g, and the average berry weight – 1.84 g, while in Kishmish belai ovalnai the values of these indices are 140-178.1 g and 1.07 g respectively. Kishmish belai krupnoiagodnai is not distributed in production vineyards – it is only grown in ampelographic collections. The commercial disadvantages of this clone include low yield, low percentage of first-class vines obtained through grafting, limited capacity for root development, etc.

The main results from the cytological investigations of the native Bulgarian vine cultivars indicate that most of them are diploid with a chromosome number 2n=38 (Table 2). Only in Rosa mena di Vacca, a cytological chimera has been found, with 2n=76 and 2n=38, but the basic chromosome number is diploid. The polyploid cells having originated as a result of endomitosis, are found single or in a group of 4-8, and sometimes even more than 10. Cells with a doubled chromosome number have been reported in the upper growth cone only in some of the studied shoots, but an entirely polyploid shoot has not been observed in any

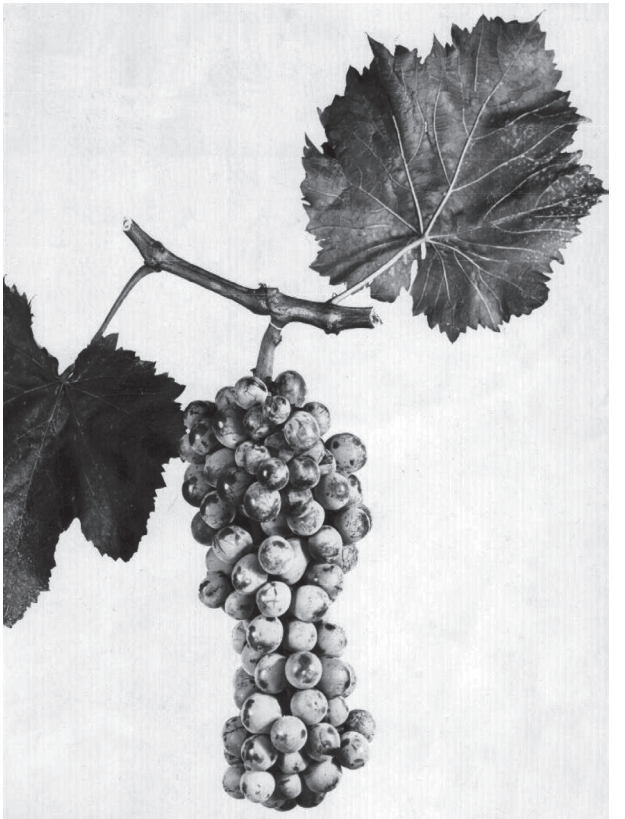

Fig. 2. Cluster from the clone Kishmish belai krupnoiagodnai with a narrowed middle section, which is a typical characteristic of some polyploid forms

Table 1
Chromosome number of the studied seedless vine cultivars

№	Cultivar	2n	№	Cultivar	2n
1.	Askery *	38	15.	Kishmish mramornai *	38
2.	Seedless hybrid VI-4	38	16.	Kishmish muskatnai	38
3.	Seedless hybrid V-6	38	17.	Kishmish bial	38
4.	Seedless late	38	18.	Kishmish rozovai *	38
5.	Kishmish white semi-seeded	38	19.	Kishmish safed okruglai	38
6.	Kishmish ashtarskii	38	20.	Kishmish sieh	38
7.	Kishmish belai ovalnai *	38	21.	Kishmish tagopskii	38
8.	Kishmish belai krupnoiagodnai *	76	22.	Kishmish tadjikskii	38
9.	Kishmish belai turkmenskii	38	23.	Kishmish uzunbashlai	38
10.	Kishmish Vatkana	38	24.	Kishmish hishrau	38
11.	Kishmish VIR	38	25.	Kishmish chernai	38
12.	Kishmish krasnai turkmenskii	38	26.	Kairmaizi Kishmish	38
13.	Kishmish kruglai	38	27.	Tarnau	38
14.	Kishmish liunda	38	28.	Korinka belaia *; Korinka chernaia *	38

* A cultivar whose chromosome number has been previously determined by other authors

Table 2
Chromosome number of the studied Bulgarian native seeded vine cultivars

№	Cultivar	2n	№	Cultivar	2n
1.	Aptish aga	38	36.	Lisicha opashka biala	38
2.	Bagrena	38	37.	Lisicha opashka chervena	38
3.	Berkovsko cherno	38	38.	Lisichina kragla	38
4.	Bolgar № 2	38	39.	Lisichina rumena	38
5.	Bolgar *	38	40.	Lisichina chervena	38
6.	Bulut uzumu	38	41.	Mavrud	38
7.	Biala debela	38	42.	Mavrud varnenski	38
8.	Bial gomas	38	43.	Marash bial	38
9.	Varna beiaza	38	44.	Marash cherven	38
10.	Vasiliko	38	45.	Mechka	38
11.	Verenichka loza	38	46.	Muskat vrachanski	38
12.	Vinenka	38	47.	Muskat dunavski	38
13.	Vinta	38	48.	Muskat trakiiski	38
14.	Galan	38	49.	Muskat cherven	38
15.	Garvan	38	50.	Morentsi	38
16.	Gomas bial (clone)	38	51.	Obichki	38
17.	Gushevitsa	38	52	Ozirovka	38
18.	Gamza	38	53.	Presedlitsa	38
19.	Gamza varnenska	38	54.	Rezakia biala	38
20.	Dimrit	38	55.	Rezakia mirizliva	38
21.	Dimiat *	38	56.	Rezakia pembiana	38
22.	Dimiat edar	38	57.	Rezakia cherna	38
23.	Dimiat cherven	38	58.	Rezakia cherna s tochitsi	38
24.	Ekshi kara	38	59.	Rosa mena di Vacca ** 76	38
25.	Zabalkanski *	38	60.	Rubin	38
26.	Zampara	38	61.	Urum izumu	38
27.	Zeinel bial	38	62.	Focha	38
28.	Zelenika	38	63.	Hora	38
29.	Zimno vineno	38	64.	Cherven septemvriiski	38
30.	Zlatanka	38	65.	Cherno tvardo	38
31.	Kabak izumu	38	66.	Shevka	38
32.	Kara gevrek	38	67.	Shiroka melnishka loza	38
33.	Keratsuda	38	68.	Iubilei	38
34.	Kehlibar	38	69.	Iulski biser	38
35.	Kokorko	38	70.	Iapladja cherna	38

Legend:
* A cultivar whose chromosome number has been previously determined by other authors.
** The figure outside the column indicates that single or grouped tetraploid cells are encountered in the cultivar.

of the cases. The karyological data on the cultivars Bolgar, Dimiat and Zabalkanski confirms previously obtained results by other authors (Todorov and Dimitrov, 1974; 1980; Krastanova, 1986).

In the world collection of viticulture literature until 1968, no cytological data regarding triploid vines is available, nor information about their commercially significant ampelographic characteristics – growth dynamics, yield, shape and size of cluster and berry, pollen sterility, ovum fertility, etc. Cytogenetic research has revealed a fundamentally new method in the selection of cultivated plants – development and utilization in agriculture of heterotic triploid forms. They are characterized by high sterility of the male and female gametophyte and low fertility. A large number of positive examples are also known in horticulture, such as triploid bananas, watermelons, sugar beet, etc. Since triploid development through self-pollination is based on accidental generation of unreduced gametes whose probability in vine is extremely low, this method has not been applied. The first triploid vines obtained through valent crosses (2n x 4n, 4n x 2n) have been created in the "Magarach" Institute (Figure 3) (Topale, 1971). During investigations into Bulgarian hybrid forms, developed by the selection researcher V. Valchev, triploid vine plants have been found with a chromosome number 2n=57 – XIX-28/4, XX-29/8 and XIX-20/48 (Figure 4). In XX-29/8 the cluster has normal size and structure, the predominating small-sized berries are seedless, while the larger berries are fewer and seeded (Figure 5). The cluster in XIX-20/48 is winged, loose, with comparatively identical in sizes and shape seedless berries (Figure 6).

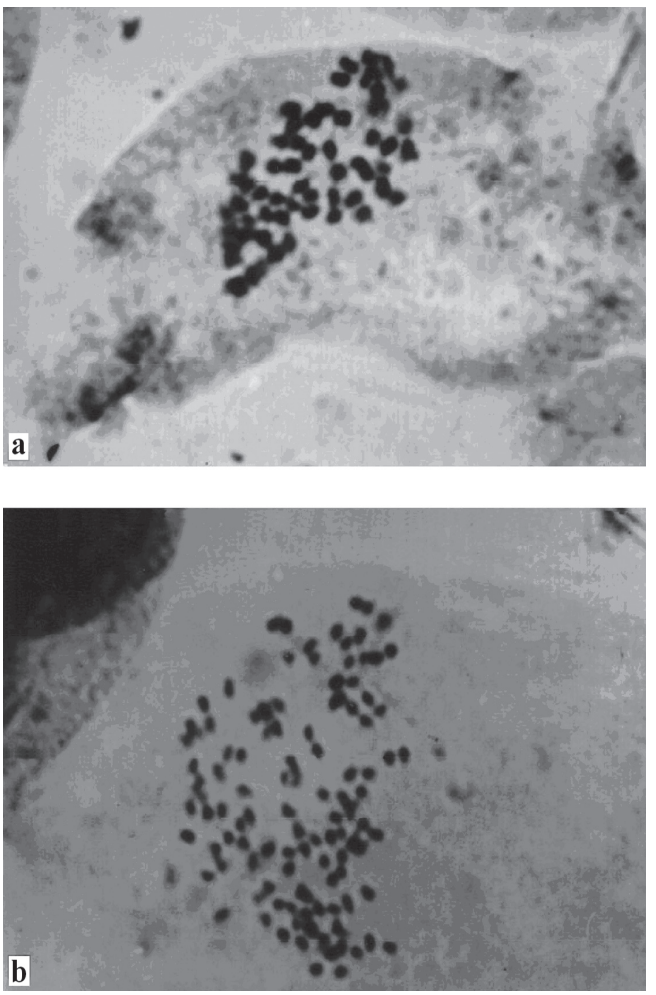

Fig. 3. Metaphase lamellae with chromosomes: a – triploid hybrid seedling (2n = 57); b –hexaploid cell – 2n=114, x 2660

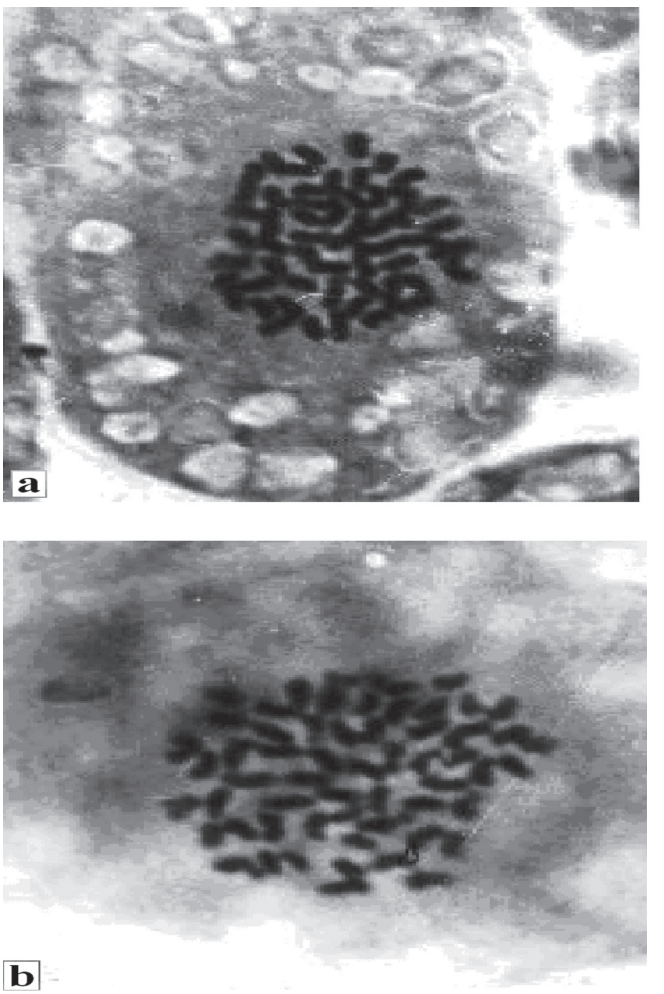

Fig. 4. Metaphase lamellae with chromosomes from triploids (2n=57): a – XX -29/8; b –XIX – 28/4, x 2660

No significant differences exist in the shape, sizes and superficial microstructures of pollen grains in the studied seedless and native vine cultivars (Figure 7). They are tricolporate, which once again indirectly confirms their diploid chromosome number.

Conclusions

In the studied seedless vine cultivars, only one polyploid with a doubled chromosome number (2n=76) has been found, while all the rest are diploid. Since triploidy (2n=57) is not one of the possible causes for the appearance of parthenocarpy in vine, seedless cultivars have most probably originated as a result of natural mutations and hybridization. The reported triploid seedlings originate from valent crosses between tetraploid (4n) and diploid (2n) cultivars. The cluster in triploid forms is comprised almost entirely of seedless berries, and the larger-sized seeded berries are extremely few.

Spontaneous polyploids or genomic mutations in vine are observed very rarely in comparison to gene mutations and recombinations, which determine the rich cultivar diversity of *Vitis vinifera* L. There are no polyploid forms among the

Fig. 5. Clusters from the triploid form XX-29/8 with 2n=57. The arrows point at normally developed berries with seeds, and all the others are seedless

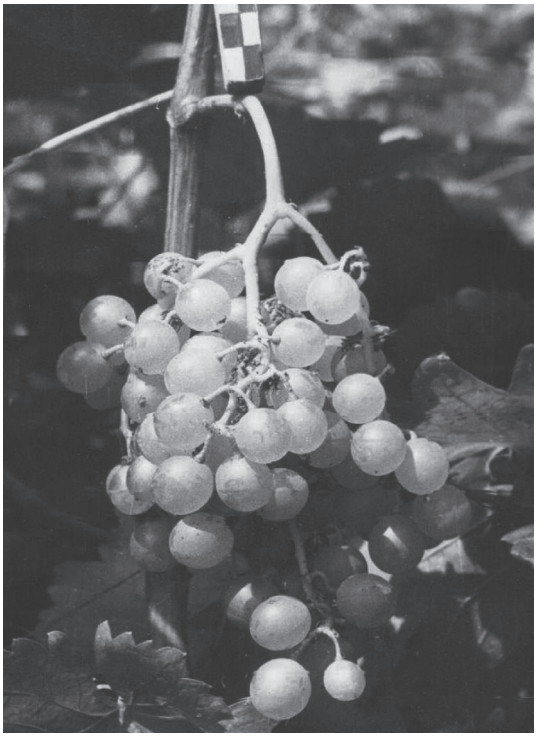

Fig. 6. Cluster from the triploid form XIX-20/48 with 2n=57

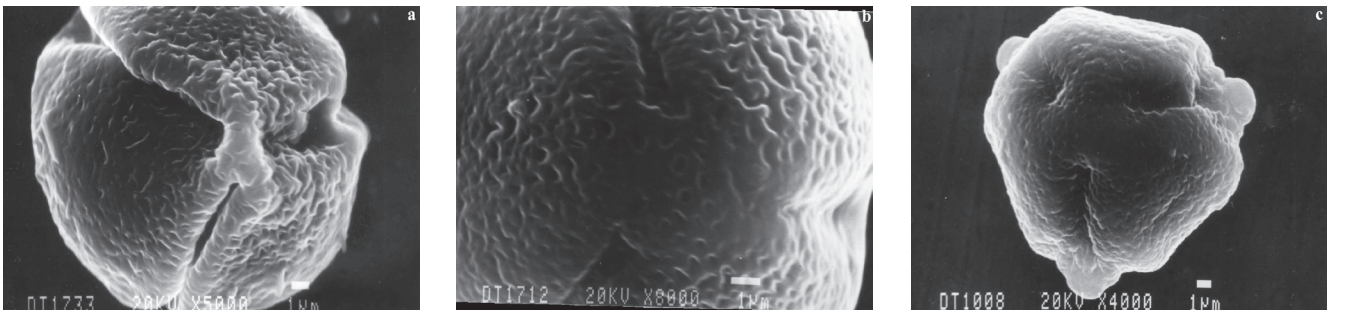

Fig. 7. Microrelief of the pollen exine in the cultivars: a - Seedless hybrid VI – 4; b - Kishmish Vatkana; c – Bolgar

researched native table and wine Bulgarian vine cultivars, in spite of their typical large-sized berries and clusters. Only a single cytochimera has been found, while all remaining 69 cultivars are diploid with a chromosome number 2n=38. The scanning electron microscope examination of pollen grains indirectly confirms the diploid chromosome number in the investigated seedless and seeded vine cultivars.

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