

Type of pollination and indices of fruit set of some Georgian grapevine varieties

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Summary

Pollen characteristics (length, width, diameter, number of pores and stain ability), type of pollination, fruit set and number of seeds per berry of the eight Georgian autochthonous grapevine varieties Rkatsiteli, Saperavi, Gorula, Gorula no. 21, Tsulukidzis Tetra, Alexandrouli, Mujuretuli and Orbeluri Ojaleshi have been investigated. It is concluded that for fruitset of the tested hermaphrodite grapevine varieties, self-pollination plays a predominant role, but in addition cross-pollination is necessary for their high fruitset.

Key words: grapevine, genotypes, fruitset, pollination, pollen-morphology, apomixis, parthenocarpy.

Introduction

There are numerous approaches on pollination of hermaphrodite grapevine varieties (*Vitis vinifera* ssp. sativa D. C.). According to ROMBOUGH (2002) one group of researchers, considers that hermaphrodite grapevine varieties are self-pollinating. Some scientists (IAKIMOV *et al.* 1977, KIMURA *et al.* 1998, GURASASHVILI and VASHAKIDZE 2004) report simultaneous self- and cross-pollination, of which self-pollination is more important. Cholokashvili and CHAKHNASHVILI (1926) and VASHAKIDZE *et al.* (1982) refer to simultaneous occurrence of both types of pollination, but depending on the genotype, self- or cross-pollination are most widespread. The aim of our investigation was to reveal the role of self- and cross-pollination in fruitset of some Georgian autochthonous grapevine genotypes. In this article the peculiarities of pollen grains, the type of pollination and fruit set of Georgian autochthonous grapevine genotypes have been studied.

Material and Methods

The Georgian autochthonous grapevine varieties Rkatsiteli, Saperavi, Gorula, Gorula no. 21, Tsulukidzis Tetra, Alexandrouli, Mujuretuli and Orbeluri Ojaleshi from the Black Sea basin (*proles Pontica* Negr.) and eastern ecological-geographical groups of *Vitis vinifera* L. (*proles Orientales* Negr.) have been explored. Cellular and morphological studies were carried out using the methods described by LAZAREVSKII (1946), PAUSHEVA (1988) and IPGRI (1997).

Studies at the cellular level were performed at the Laboratory of Cytogenetics of the Georgian Scientific-Re-

search Institute of Horticulture, Viticulture and Oenology (Tbilisi) in 2003-2004.

Pollen morphology and parameters like length, width and diameter of 50 fresh pollen grains have been investigated by ocular-micrometer under airborne conditions.

Pollen fertility was investigated by the acetocarmine method, including fixation of anthers in Karnua's solution 3:1 (ethanol : acetic acid) for 30 min; three times washing and storage in 75 % ethanol. The pollen grains were stained by 1 % acetocarmine; 300 pollen grains of each accession have been tested using temporal cytological preparation. The amount of stained (fertile) and unstained (sterile) pollen were counted.

Pores of about 100 pollen grains were studied on a temporal preparation using a mixed solution of acetic acid and sulfuric acid. The number of tricolporate grains was counted. Microscopic analysis was carried out by a MBI-3 and a PZO microscope.

To examine the type of pollination characteristic for the above-mentioned genotypes experimental plots of the Georgian Scientific-Research Institute of Horticulture, Viticulture and Oenology were used; vines were classified according to the following scheme: self-pollination, free-pollination, and apomixis.

In case of free- and self-pollination, the indices of fruit set and the number of seeds in berries were determined using 10-30 inflorescences and bunches, respectively. During self-pollination, the inflorescences were enclosed by paper bags two weeks before bloom; the indices of fruit set were counted two weeks after bloom.

In berries obtained from free- and self-pollination, the shares of seedless, 1, 2, 3, 4 and more seeded berries were calculated.

To detect apomixis ability, the inflorescences were enclosed in paper bags two weeks before bloom, flowers were emasculated 5-6 d before bloom and the indices of fruit set were counted two weeks after bloom.

The results were statistically processed according to LAKIN (1990).

Results

According to KHARITONASHVILI (1971) and Vashakidze and GURASASHVILI (1999) meiosis occurs in the mother cells of the cvs Rkatsiteli, Saperavi, Gorula, Gorula no. 21, Tsulukidzis Tetra, Alexandrouli, Mujuretuli and Orbeluri Ojaleshi. The tetrads are formed by simultaneous type. Within tetrads microspores are arranged with tetrahedral type.

The vegetative nucleus of binuclear pollen grains of grapevine has an oval shape and reveals weak reaction to DNA. The generative nucleus is of lense shape and characterized with Fiolgen positive reaction. Sperm cells of grapevines are of oval elongated shape and characterized with insignificant cytoplasm.

According to KHARITONASHVILI (1971), IAKIMOV *et al.* (1977), VASHAKIDZE and GURASASHVILI (1999), GURASASHVILI and VASHAKIDZE (2004) bisexual grapevine varieties are characterized by small, wheat grain shaped normal pollen grains in airborne conditions. After wetting or on artificial nutrient substrates, they were gradually swollen first having a triangular shape and finally a spherical shape.

The genotypes under investigation, basically have tricolporate pollen grains, of which the polar axis length under airborne condition varies from 28.4 to 35.0 μm , the length of equatorial axis from 14.7 to 17.2 μm and diameter (stained with acetocarmine) varied from 18.1 to 24.4 μm (Fig. 1). Maximal values were obtained for cv. Orbeluri Ojaleshi, minimal values for Saperavi. The coefficient of variance is small for Saperavi, Gorula, Gorula no. 21, Tsulukidzis Tetra and Orbeluri Ojaleshi ($Cv < 10\%$) and comparatively high for Rkatsiteli, Alexandrouli and Mujuretuli ($10 < Cv < 15\%$).

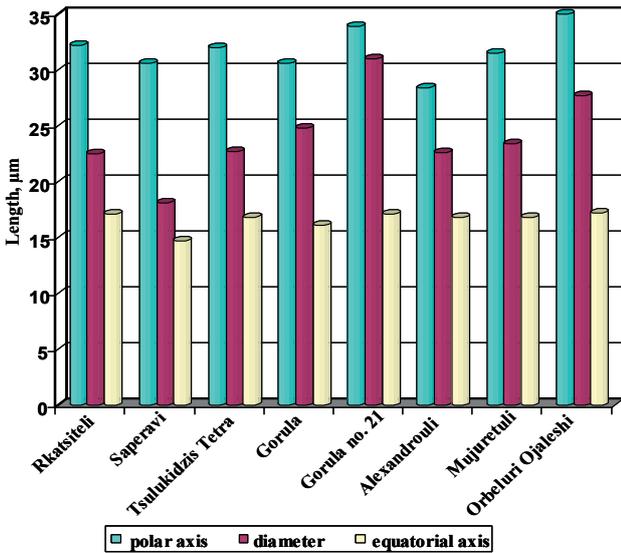


Fig. 1: Length of polar and equatorial axes and diameter of pollen grains.

The indices of pollen staining in tested genotypes are high: 70.0 to 98.8 % (Fig. 2). According to these indices, they are divided into two groups:

1. Genotypes characterized by very high pollen staining ability ($P > 85\%$): Rkatsiteli, Saperavi, Tsulukidzis Tetra, Gorula and Orbeluri Ojaleshi.
2. Genotypes characterized by high pollen staining ability ($70\% < P < 85\%$): Gorula No. 21, Alexandrouli and Mujuretuli.

The relative content of tricolporate pollen grains varies between 67.6 and 88.1 % (Fig. 2) and due to this they are divided into two groups with similar sequences as in the case of pollen staining. These results show that pollen grains of the above were available for fruitset.

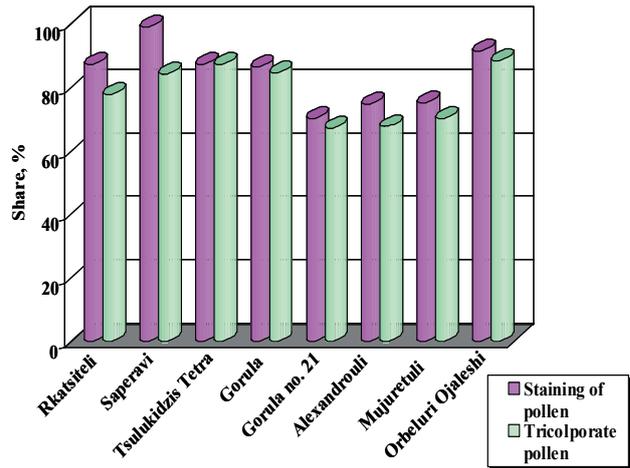


Fig. 2: Staining and share of tricolporate pollen grains.

The indices of fruitset by self-pollination differ between the varieties ranging from 16.9 % to 61.9 % (Tab. 1). Within genotypes the indices of fruitset are different between self- and free-pollination. Particularly, the indices of fruitset of Rkatsiteli, Alexandrouli and Tsulukidzis Tetra after free-pollination are higher than after self-pollination, while for Saperavi, Gorula, Gorula no. 21, Mujuretuli they are lower. The type of pollination does not affect fruitset of Orbeluri Ojaleshi. The influence of genotypes on the fruitset are significant $p < 0.05$ (two factor dispersal analysis), while the influence of the type of pollination is not significant.

There is a potential to develop 4 seeds in grapevine berries, but due to the aberrations during microsporogenesis, macrosporogenesis, embryogenesis, and peculiarities of genotypes, 0, 1, 2, 3, 4 and more seeds may occur in berries. The number of seeds and the ability for parthenocarp were studied with Rkatsiteli, Saperavi, Gorula, Gorula no. 21 and Tsulukidzis Tetra. The influence of the pollination type on the development of seeds in berries, with checking c^2 -criteria, has shown that the difference is not significant for Rkatsiteli (Tab. 2), while, it is significant for Saperavi, Gorula, Gorula no. 21 and Tsulukidzis Tetra. In these genotypes, in case of self-pollination, compared to free pollination, the number of parthenocarpic berries was increased; the number of one-seeded berries was also increased, while the number of two-, three- and four-seeded berries was reduced. The number of seeds in 100 berries, after self-pollination, was reduced compared to free-pollination, too.

The number of seedless parthenocarpic berries after self-pollination was small (3.5-7.3 %) in Saperavi, Gorula and Gorula no. 21; their share was increased in Tsulukidzis Tetra from 3.0 % to 6.5 % compared to free-pollination. This phenomenon was not observed in Rkatsiteli.

The tests carried out to define apomixis ability of the above-mentioned genotypes evidenced that this phenomenon is not a characteristic feature. Particularly, fruitset has not been observed for the emasculated 4048 flowers of Saperavi, the 5132 flowers of Rkatsiteli, the 2740 flowers of Gorula, the 1517 flowers of Alexandrouli and the 1733 flowers of Mujuretulu in many years of investigation.

Table 1

Indices of fruit-set after free- and self-pollination

Variety	Type of pollination	Number of inflorescences	Total number of flowers	Number of flowers per inflorescence	Index of fruit set		P
					n	P±SD (%)	
Rkatsiteli	Free	10	1984	198.4	1229	61.9 ± 1.1	0.001
	Self	10	2548	254.8	1411	54.7 ± 1.0	
Saperavi	Free	10	3737	373.7	1673	44.8 ± 0.8	0.001
	Self	10	1377	137.7	1256	91.2 ± 0.8	
Gorula	Free	25	5147	205.9	1357	26.4 ± 0.6	0.001
	Self	25	4903	196.4	1524	31.1 ± 0.7	
Gorula no. 21	Free	25	5255	210.2	1380	26.3 ± 0.6	0.001
	Self	25	5042	202.5	1808	35.9 ± 0.7	
Tsulukidzis Tetra	Free	25	5211	208	1487	28.5 ± 0.6	0.001
	Self	21	3423	163	805	23.5 ± 0.7	
Alexandrouli	Free	20	3026	151	606	20.0 ± 0.7	0.001
	Self	22	2585	117.5	337	13.3 ± 0.7	
Mujuretuli	Free	30	6116	203	1036	16.9 ± 0.5	0.001
	Self	20	2310	115	622	26.9 ± 0.9	
Orbeluri Ojaleshi	Free	24	2999	125	1521	50.7 ± 0.9	-
	Self	18	2306	128	1169	50.7 ± 1.0	

Table 2

Shares of 0-, 1-, 2-, 3- and 4-seeded berries

Variety	Type of pollination	Total number of berries	Number of seeds in berries					Number of seeds in 100 berries	p
			0	1	2	3	4		
Rkatsiteli	Free	2650	0	55.1 ± 1.0	37.9 ± 0.9	6.2 ± 0.5	0.1	149.9	-
	Self	3109	0	55.7 ± 1.0	36.7 ± 0.9	6.2 ± 0.4	0.2	148.5	
Saperavi	Free	3009	0.1	61.0 ± 0.9	33.2 ± 0.9	5.6 ± 0.4	0.1	144.6	0.01
	Self	3795	7.3 ± 0.4	72.2 ± 0.7	18.4 ± 0.6	1.9 ± 1.2	0.1	114.7	
Gorula	Free	1510	0	67.9 ± 1.2	29.7 ± 2.2	3.4 ± 0.5	0	137.5	0.01
	Self	913	3.5 ± 0.6	74.9 ± 1.7	20.3 ± 1.3	1.4 ± 0.4	0	119.7	
Gorula no. 21	Free	1562	0.1	68.0 ± 1.4	29.3 ± 1.2	2.5 ± 0.4	0	134.1	0.01
	Self	697	3.7 ± 0.7	75.3 ± 1.6	19.7 ± 1.5	1.3 ± 0.4	0	118.5	
Tsulukidzis Tetra	Free	1212	3.0 ± 0.5	67.5 ± 1.3	26.7 ± 1.3	2.0 ± 0.4	0	126.7	0.05
	Self	444	6.5 ± 1.2	77.9 ± 20	14.6 ± 1.7	1.6 ± 0.6	0	111.9	

Discussion and Conclusion

According to the results obtained from morphological and staining studies, the pollen grains of the genotypes Rkatsiteli, Saperavi, Gorula, Gorula no. 21, Tsulukidzis Tetra, Alexandrouli, Mujuretuli and Orbeluri Ojaleshi, are able for fruitset and the data may be used as additional micro-structural markers to identify these varieties

The factor genotype affects significantly the indices of fruit-set of tested varieties. It is lowest for Mujuretuli (16.9 %), and highest for Rkatsiteli (61.9 %). The variability of fruitset as affected by the pollination type depends on the genotype, too. Particularly, after free-pollination the

indices of fruit-set are higher for Rkatsiteli, Tsulukidzis Tetra and Alexandrouli than after self-pollination, while for Saperavi, Gorula, Gorula no. 21 and Mujuretuli indices are lower, and for Orbeluri Ojaleshi no difference is found. Thus, the main type of pollination is self-pollination for Rkatsiteli, Tsulukidzis Tetra and Alexandrouli, but cross-pollination occurs simultaneously with self-pollination and its additional action is necessary to achieve a high index of fruitset.

Checking χ^2 -criteria, the number of seeds in berries of Rkatsiteli, Saperavi, Gorula, Gorula no. 21 and Tsulukidzis Tetra, obtained after self- and free-pollination, revealed that the type of pollination does not affect this feature of

Rkatsiteli. However, in the case of Saperavi, Tsulukidzis Tetra, Gorula and Gorula no. 21 after self-pollination, seedless, parthenocarpical berries developed, and the share of one-seeded berries increased, while the percentage of two-, three- and four-seeded berries decreased. For these genotypes, indices of the fruitset were higher after self-pollination than after free-pollination, but the number of seeds in berries was lower. Thus we assumed that additive influences, *i.e.* small shares of cross-pollination and simultaneous self-pollination are characteristic for them.

Our investigation on fruitset of Georgian grapevine varieties indicates: For Rkatsiteli, Saperavi, Gorula, Gorula no. 21, Tsulukidzis Tetra, Alexandrouli, Mujuretuli and Orbeluri Ojaleshi and clone Gorula no. 21, self-pollination dominates. However, in addition cross-pollination is necessary.

References

- CHOLOKASHVILI, S.; CHAKHNASHVILI, N.; 1926: Results of a study on Georgian grapevine. *Agron. Proc. Georgian Exp. Tbilisi. Georgia* **3**, 17-19.
- GURASASHVILI, V.; VASHAKIDZE, L.; 2004: Type of pollination and frequent of fruit-set of Georgian grapevine variety Gorula. *J. Probl. Agric. Sci. Tbilisi. Georgia* **26**, 72-75.
- IAKIMOV, L. M.; LITVAK, A. I.; BALANY, G.; 1977: Atlas of Embryology of Grapevine. Shtnitsa Publ., Chisinau, Moldova.
- IPGRI Descriptors for Grapevine (*Vitis* spp), 1997, Rome.
- KHARITONASHVILI, L.; 1971: Cytoembryological Study of some Georgian Grapevine Varieties. State Univ. Publ., Tbilisi, Georgia.
- KIMURA, P. H.; OKAMOTO, C.; HIRANO, K.; 1998: The mode of pollination and stigma receptivity in "*Vitis coignetiae* Pull.". *Am. J. Enol. Vitic.* **49**, 1-5.
- LAKIN, G. F.; 1990: Biometrics. *Visshaia Shkola*, Minsk, Belarus.
- LAZAREVSKI, M. A.; 1946: The Methods of Grapevine Description. *Ampeology of the USSR. Vol. 1. Pishepronizdat Publ., Moscow.*
- NEGRUL, A. M.; 1936: *Genetical Foundation of Grapevine Breeding.* St. Petersburg. Russia.
- PAUSHEVA, Z. P.; 1988: *Methodology of plant cytology.* Kolos Publ., Moscow.
- ROMBOUGH, L.; 2002: *The grape grower.* Chelsea Green Publ., Vermont, USA.
- VASHAKIDZE, L.; GOGIAVA, E.; BEJUASHVILIU, N.; 1982: The Biological Peculiarities of some Georgian Grapevine Varieties by Breeding. Conf. "Productivity of Subtropical Crops". Maxaradze-Anaseuli. Georgia.
- VASHAKIDZE, L.; GURASASHVILI, V.; 1999: Process of microsporogenesis of the Georgian grapevine variety Gorula and its clone no. 21. *J. Vazi da Ghvino* **3**, 74-82.

Received October 13, 2005