Vitis **54** (Special Issue), 175–176 (2015)

Research Note

Cytoembryological and morphometric characteristics of some Armenian grape cultivars

A. Nebish[1,2,3], G. Melyan[1] and R. Aroutiounian[1,2]

[1] Armenian Academy of Viticulture and Wine-making, Yerevan, Armenia
[2] Yerevan State University, Faculty of Biology, Department of Genetics and Cytology, Yerevan, Armenia
[3] Research group of Plant Genetics and Immunology, Institute of Molecular Biology of National Academy of Sciences, Yerevan, Armenia

K e y w o r d s : Caucasus; pollen; ovule; carpological descriptors.

Introduction: The conservation and phenotypic characterization of Armenian grapevine gene pool is the basis for its application in future breeding programs. The cytoembryological analysis and the phenotypic characterization of grapevine male and female gametophytes and of early developed embryos could improve the understanding of genetic mechanisms involved at different stages of reproductive development (Pratt 1971, Mullins *et al.* 1992, Fernandes *et al*. 2007, Nebish 2012). Flowering and berry set are important stages of grapevine development, and they could also affect the yield quantity and quality (Longbottom 2007).

Grapevine cultivars are highly heterozygous. They show a large variation in inflorescence size, berry size, shape, weight and colour depending on genotypes and environmental conditions (This *et al.* 2006, Carmona *et al.* 2008).

The aim of our study was the phenotypic characterization of some Armenian grape cultivars by cytoembryological and carpological analysis.

Material and Methods: Ten accessions of Armenian grapevines were analysed including: 5 *Vitis vinifera* L. cultivars (authochthonous 'Mskhali', 'Garan dmak' and clone of 'Ararati' cultivars, 'Tokun' - open pollination of 'Spitak Arakseni', 'Muscat haykakan' - open pollination of 'Muscat cherniy'); 3 intraspecific hyvrids ('Berkanush', 'Parvana' and 'Erebuni') and 2 *V. vinifera* x *V. amurensis* interspecific hybrids ('Charentsi' and 'Meghrabuyr'). All the accessions were grown in the same collection of the Scientific Center of Viticulture, Fruit-Growing and Wine-Making (Merdzavan, Armenia). In 2013 flowers for cyto-embryological analysis of micro- and macro-gametophyte, and ripe berries for morphometric studies were collected. For estimation of pollen fertility the acetocarmine staining method have been used. Pollen sterility data were obtained based on the analysis of about 5000 pollen grains for each accession. Concerning cytoembryological investigations, fixation in FAA fixative (formaldehyde – acetic acid – ethanol) were applied. Material was then stained by Mayer's hematoxylin and eosin (H&E) (Bancroft and Gamble 2008). Permanent slides with paraffin-embedded flower's thin sections (8-10 μm) were analyzed using a Motic 10 digital microscope. Morphometric features were described on digital photos of ripe berries according to the IPGRI, OIV and UPOV phenotypic descriptors (International Plant Genetic Resources Institute; 1997) by the ImageJ software. Statistical analysis of data was carried out using Student's t-test and 5 % probability was considered significant.

Results and Discussions: The results demonstrated a high level of pollen fertility in the investigated grapevine cultivars (Table). The highest level of pollen fertility were recorded in 'Mskhali', 'Tokun' and 'Charentsi', and the lowest were found in 'Garan dmak' and 'Ararati' (less than 70 %). Low levels of viable pollen or ovules lead to low fertilization and abnormal seeds development. The pollen abortion reflects deletions in part of the genome (Gilles and Prakash 1987). At the same time, abnormalities in female gametophyte development were registered. Some ovules in the ovaries were not developed or resulted degenerated. In the aborted ovules, the embryo sac and the nucellus with

T a b l e

Cytoembryological and morphometric characteristics of Armenian grape cultivars

Cultivar	Pollen fertility (%)	Number of ovules per flower	Number of seeds per berry	Berry weight (g)	Berry size	
					length (mm)	width (mm)
Mskhali	94.00 ± 0.11	0.91 ± 0.10	0.54 ± 0.06	1.88 ± 0.15	16.59 ± 2.01	14.93 ± 1.80
Garan dmak	65.80 ± 0.45	1.28 ± 0.06	1.23 ± 0.04	1.67 ± 0.07	14.81 ± 1.79	14.62 ± 1.77
Ararati clone	69.55 ± 0.46	0.86 ± 0.09	0.54 ± 0.08	5.51 ± 0.31	22.50 ± 1.81	20.12 ± 1.35
Tokun	93.90 ± 0.11	1.34 ± 0.08	1.07 ± 0.06	1.38 ± 0.05	16.87 ± 1.51	14.61 ± 1.20
Muscat haykakan	87.30 ± 0.22	2.14 ± 0.08	1.99 ± 0.13	1.15 ± 0.14	14.62 ± 1.39	13.86 ± 1.25
Berkanush	87.88 ± 0.21	2.38 ± 0.08	1.93 ± 0.10	1.80 ± 0.11	14.26 ± 1.03	13.05 ± 1.23
Parvana	85.96 ± 0.24	1.82 ± 0.07	0	3.91 ± 0.16	24.43 ± 2.47	20.99 ± 2.22
Erebuni	82.23 ± 0.28	2.82 ± 0.10	2.38 ± 0.06	5.63 ± 0.16	25.02 ± 3.56	23.98 ± 2.57
Charentsi	97.56 ± 0.14	2.46 ± 0.10	1.68 ± 0.08	2.48 ± 0.09	17.61 ± 1.92	17.33 ± 3.96
Meghrabuyr	89.90 ± 0.18	1.71 ± 0.10	1.54 ± 0.07	1.93 ± 0.09	16.54 ± 1.39	16.04 ± 1.48

Correspondence to: Dr. A. Nebish, Department of Genetics and Cytology, Faculty of Biology, Yerevan State University, Alex Manoogian street 1, Yerevan, 0025, Armenia, E-mail: armeno_a@rambler.ru

the inner integument cells were wrinkled and separated from the outer integument of the ovule. For each investigated sample, 0.86-2.46 ovules per flower were detected. Minimal numbers of ovules with embryo sac per flower were found in 'Mskhali' and 'Ararati' (respectively 0.91 ± 0.10 and 0.86 ± 0.09). In the intra- and interspecific crosses the highest number of ovules ranged between 1.71 ± 0.10 in 'Meghrabuyr' and 2.46 ± 0.10 in 'Charentsi'. The number of ovules per flower in autochthonous and crossed accessions resulted to be significantly different ($P < 0.008$).

During fruit set, only part of the developed ovules produced seeds with both embryo and cellular endosperm. The percentage of developed seeds per berry was higher in 'Garan dmak', 'Muscat haykakan' and 'Meghrabuyr', then in 'Mskhali' and 'Ararati'. In the stenospermocarpic table cultivar 'Parvana', only berries with undeveloped small seeds without embryo and endosperm were registered.

The reasons of the low recorded number of seeds per berry could be related to: abnormalities in ovule development, low level of pollen fertility, abortion of some ovules after fertilization, insufficient pollination and fertilization. The same effects were also described by BARRITT (1970), EBADI *et al.* (1996) and ODABAS *et al.* (2007). The abnormalities in reproductive development of grapes can have genetic origin or they can be caused by adverse environmental conditions (OLMO 1980). Perhaps, the significant differences in ovule development between autochthonous and crossed cultivars could result from extended vegetative reproduction, that caused the loss of the normal development of generative system.

Berry morphometric data recorded average weights ranging between 1.15 and 5.63 g; and average size variations between 13.05 mm and 25.02 mm. The largest berries were developed in 'Erebuni' and 'Ararati' table cultivars with average weight of 5.5 g, and size higher than 20 mm. The berries of 'Parvana' were characterized by average weight of 3.91 ± 0.16 g, length 24.43 ± 2.47 mm and width 20.99 ± 2.22 mm. In wine cultivars the values of morphometric traits were lower (average weight of berries from 1.15 to 2.48 g and sizes from 13.05 to 17.61 mm). The smallest berries were produced in 'Muscat haykakan' with average weight of 1.15 ± 0.14 g, length 14.62 ± 1.39 mm and width 13.86 ± 1.25 mm.

There were no significant differences in berry weight and sizes between autochthonous and crossed cultivars. As expected, berries weight and sizes were higher for table grapes, than in wine cultivars ($P < 0.05$).

Conclusions: The results obtained demonstrated abnormalities during both pollen and ovule development. Significant differences were found only for number of ovules per flower between autochthonous and crossed cultivars. However, no differences in berry morphometric data were recorded between autochthonous and crossed cultivars.

Cytoembryological and morphometric information could be applied as breeding markers to improve local viticulture and winemaking industry. Our data will be useful for target table grapes selection of stenospermocarpic cultivars for production of large berries with small seeds.

Joint publication of the COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding".

BANCROFT, J. D.; GAMBLE, M.; 2008: Theory and Practice of Histological Techniques. 6th ed. Churchill Livingstone Elsevier, Philadelphia, PA.

BARRITT, B. H.; 1970: Ovule development in seeded and seedless grapes. Vitis **9**, 7-14.

CARMONA, M. J.; CHAIB, J.; MARTIEZ-ZAPATER, J. M.; THOMAS, M. R.; 2008: A molecular genetic perspective of reproductive development in grapevine. J. Exp. Bot. **59**, 2579-2596.

EBADI, A., SEDGLEY, M.; MAY, P.; COOMBE, B. G.; 1996: Seed development and abortion in *Vitis vinifera* L., cv. Chardonnay. Int. J. Plant Sci. **157**, 703-712.

FERNANDEZ, L.; TORREGROSA, L.; TERRIER, N.; SREEKANTAN, L.; GRIMPLET, J.; DAVIES, C.; THOMAS, M. R.; ROMIEU, C.; AGEORGES, A.; 2007: Identification of genes associated with flesh morphogenesis during grapevine fruit development. Plant Mol. Biol. **63**, 307-323.

GILES, L. K.; PRAKASH, P. J.; 1987: Pollen cytology and development. Int. Rev. Cytol. **5**, 107-151.

INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE; 1997: Descriptors for Grapevine (*Vitis* spp). IPGRI, Rome, Italy.

LONGBOTTOM, M. L.; 2007: The reproductive biology of grapevines – factors that affect flowering and fruit set. PhD Thesis, Univ. Adelaide, Australia.

MULLINS, M. G.; BOUQUET, A.; WILLIAMS L. E.; 1992: Sexual propagation. In: Biology of the Grapevine, 61-79. Cambridge Press.

NEBISH, A. A.; 2012: Investigation of cellular development and embryogenesis in Armenian grapevine varieties. International young scientists conference "Perspectives for development of molecular and cellular biology - 3", 170-174. Yerevan, Armenia.

ODABAS, F.; BEYHAN, N.; CELIK, H.; 2007: The Effect of GA3 and CCC on Ovule and Seed Development In Kabuguyufka (*Vitis vinifera* L.). Int. J. Agric. Res. **2**, 145-151.

OLMO, H. P.; 1980: Selecting and breeding new grape varieties, 23-24. California Agricultural University.

PEDERSON, S.; SIMONSEN, V.; LOESCHCKE, V.; 1987: Overlap of gametophyte and sporophytic gene expression in barley. Theor.Appl. Genet. **75**, 200-206.

PRATT, C.; 1971: Reproductive Anatomy in Cultivated Grapes, Am. J. Enol. Vitic. **22**, 92-109.

THIS, P.; LACOMBE, T.; THOMAS M. R.; 2006: Historical origins and genetic diversity of wine grapes. Trends Genet. **22**, 511-519.