EXAMINING THE STATUS CYTOGENETIC ON SOME AUTOCHTHONOUS VARIETIES A GRAPEVINE IN R. MACEDONIA ACCORDING O.I.V. SYSTEM

Biljana Korunoska[1], Zvonimir Bozinovic[2] , Klime Beleski[1], Krum Boskov[2], Zlatko Prculoski[2], Frosina Trajanovska[1]

[1]University „Ss.Cyril and Methodius, Institute of Agriculture, Skopje, R. Macedonia
[2]University „Ss.Cyril and Methodius“, Faculty of Agricultural Sciences and Food, Skopje, R. Macedonia

Corresponding author: markovskabiljana@yahoo.com

Abstract
In R. Macedonia has many domestic (autochthonous) or domesticated varieties of vines. Many of them are similar to some varieties of from neighboring countries, and some of them are very different between them, and are also differ from the other varieties. It depends on heritable traits of their ancestors and their origins, from the centers of origin. They represent undiscovered source of many genes that are carriers of positive properties and predominantly transmitted to future generations. Many of them are unidentified and not known their exact origin. Therefore efforts are made with certain adequate methods to identify (ampelographic, ampelometric, DNA identification).In our research we covered several table and wine varieties of grapevine from different vineyards through R. Macedonia. In the trials used methods for determination of cytogenetic status according O.I.V. system of descriptors - (number of chromosomes, ploidy level, germination of pollen, meiosis, type and characteristics of the flower, etc.). We used statistical computer processing by (SPSS) program.Obtained are interesting results in terms of the structure of the flower and the cell division that indicate the similarity between them and their common origin.

Keywords: autochthonous varieties, description, cytogenetic status, meiosis.

Introduction

In this paper, 3 autochthonous (domestic) or domesticated varieties of grapevine and 1 muscat variety for comparison from the same group, different subgroup were examined. The main objective of this study is to investigate the cytogenetic status of varieties of the same center of origin. Although variability in polyploid and chromosomes (tetraploidy and aberrations) in muscat varieties, the autochthonous varieties and muscat varieties grown in the Republic of Macedonia have been shown to have stable and constant cytogenetic status. Often in tested varieties of grapevine used the term domesticated varieties of grapevine, not autochthonous or domestic, because the Balkans for many years have introduced many varieties of grapevine in the center of origin of the Middle East and they were mixed with domestic species of the genus *Vitis.* Therefore it is not known with precision their genetic origin. In these trials, cytogenetic status testing methods were used only to the level of karyotype analysis (chromosome counts and measurements) where no significant differences in the chromosomal structure in the nucleus were observed. These significant differences can be verified in the processing of the genomic structure and certain genes, and it is therefore necessary to go to the DNA analysis, which would identify the similarities between the DNA fragments that are responsible for the similarities between varieties and similar centers of origin.

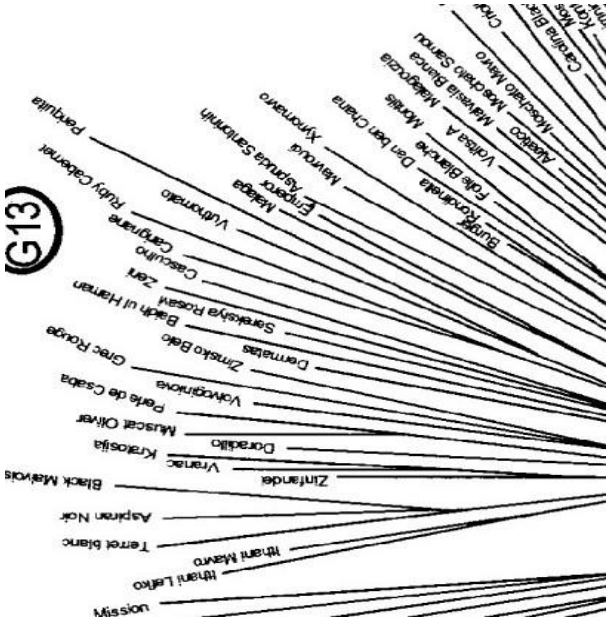
Material and methods

For examination of mitosis and meiosis in the varieties under the microscope, it is necessary prior germination of the seeds of the examined grape cultivars and the examined crossing combination, which is performed by keeping the seeds in isolated plates, slightly covered with water, alternating

6 hours on a temperature of -2°C to -3°C, in a refrigerator and on 25°C in a thermostat or a room temperature next to some heater. After the germination of the seeds they were slightly dried and planted in pots filled with garden soil and bio-fertilizer. For examination of the mitosis at the grape varieties, germinated roots 5-10 mm long were used.

- Monastery white (Klis üzum) - white table variety, with belonging to eastern varieties (convarietas orientalis, subconvarietas antasiatica), in our country found in Gevgelija-Valandovo vineyard region.
- **Stanusina** - black wine variety with belonging to Black Sea - Balkan varieties (convarietas pontica, subconvarietas balcanica), in our country found in Tikveš, Ohrid, Bitola, Štip vineyard region.
- Kratosija - black wine variety with belonging to Black Sea - Balkan varieties (convarietas pontica, subconvarietas balcanica), in our country found in Montenegro, Dalmatia, Herzegovina, Macedonia vineyard region.
- **Temjanika** - white wine variety with belonging to Black Sea - Balkan varieties (convarietas orientalis, subconvarietas caspica), in our country found in Tikveš, vineyard region

The examinations were performed in several successive years from 2006 to 2016, in Ohrid, Tikveš, Gevgelija-Valandovo and Skopje vineyard region (7). Among the results is used statistical processing according (SPSS) program. A special computer program for measuring chromosomal parameters and for the preparation of the karyotype and vine cariogram was used. A special microscope with a computer program with measuring units for measuring chromosomes in micrometers (μm) was also used. The cytogenetic status of varieties are examined according with description lists of primary and secondary OIV Code descriptors. The list was proposed by the EU-PROJECT GENRES 081 - 09/2001. Addition- ally recent edition of 2009 was used.



Dendogram 1. Aradhya, M. and col. 2013

Results and discussion

The examined varieties - monastery white (klis üzum), stanusina, kratosija and temjanika belong to the sub-genus Euvitis and they are diploid cultivars (2n = 38).

Cytological status was examined according to the pattern O.I.V.

12. Cytological characters
12.1 Chromosome number
12.2 Ploidy level; (e.g. aneuploid or structural rearrangement)
12.3 Pollen viability; Specify the method i.e. germination in a solution or grain staining
12.4 Meiosis chromosome associations; Mean of 50 microspore mother cells,

observed during metaphase
12.5 Other cytological characters; (e.g. stomata density and size)
13. Identified genes
Describe any known specific mutant present in the accession
The germination of the pollen was examined *in vitro*, with planting pollen grains in fertile base of 15% saccharose solution, in a preparation hanging drop and with keeping it in a thermostat on a temperature of 21ºC. After that the germinated pollen grains were counted and photographed under a microscope [2].
Fertilization, as part of the genetic status, was examined by determining the self - fertilization (autogamous) and cross - fertilization (ksenogamous). The observation and counting the chromosomes of the examined varieties and of the grapevines in general, is very difficult because they are tiny, their number is great and it is also very difficult to differentiate them from the cytoplasm. The cytoplasm is very thick, it blocks the dispersion of the chromosomes, so they concentrate on one place and can not be macerated during the preparation. With that, the contrast and the clarity of the sight under the microscope is lost [2], [6]. Tables 1, 2 and 3 and Chart 1 show the diploid number of chromosomes according to the standard scheme and the length of chromosomes in micrometers (μm**)** with statistical differences between individual chromosomes. Not observed any more concessions and abnormalities in metaphase chromosomes in the different cultivars [2], [6], [8]. On figures 1 and 2, chromosomes of grape varieties are shown. At the examined grape varieties – monastery white (klis üzum), stanusina, kratosija and temjanika, the metaphase is normal, there are no anomalies in the structure and the number of chromosomes, there are 38 clearly differentiated chromosomes under microscope. The dispersion of the chromosomes is slightly bigger and the cytoplasm is more porous in comparison with the wine grapes. For that reason there is better separation of the chromosomes, and they look bigger and clearer.[3], [4], [5].

Table 1. Chromosome constitutions in normally diploid organism with 2*n* = 38 chromosomes (labeled A, B, and C) in the basic set

Monastery white, Stanusina, Kratosija Temjanika	Designation	Constitution	Number of chromosomes
Monoploid	*n*	ABC	19
Diploid	2*n*	AABBCC	38
Triploid	3*n*	AAABBBCCC	57
Tetraploid	4*n*	AAAABBBBCCCC	76
Monosomic	2*n* − 1	ABBCC	37
		AABCC	37
		AABBC	37
Trisomic	2*n* + 1	AAABBCC	39
		AABBBCC	39
		AABBCCC	39

The best fertilization showed Temjanika variety, best germination of pollen showed white variety Monastery and lowest fertility slightest germination of pollen showed the variety Kratosija. [8]. Insignificant abnormalities in fertilization have been shown in the Kratosija variety, because we observed certain abnormalities in the meiotic chromosomes at the fertilization stage in the flower. So sometimes in this variety is less germination of pollen and less fertilization. According to Aradhya,

M. and col. 2013, which have prepared a map for several varieties of vines, mostly for varieties from Europe and Asia), the investigated varieties in our work are in the Ghetto 13 (along with varieties Vranec, white winter and Muscat Oliver) [1], [7].

Table 2. Length of chromosomes in tested cultivars and statistical processing

Haploid number of chromosomes N°	Vitis Vinifera subsp. Vinifera code Vvi_v 2n = 38			
	Monastery white	Stanusina	Kratosija	Temjanika
	µm	µm	µm	µm
01	1,69	1,68	1,67	1,70
02	1,57	1,58	1,56	1,63
03	1,50	1,49	1,48	1,51
04	1,41	1,39	1,37	1,42
05	1,36	1,35	1,34	1,37
06	1,33	1,30	1,27	1,30
07	1,29	1,28	1,25	1,25
08	1,26	1,25	1,22	1,22
09	1,21	1,20	1,19	1,19
10	1,18	1,18	1,16	1,17
11	1,16	1,15	1,14	1,12
12	1,13	1,12	1,11	1,11
13	1,10	1,10	1,09	1,08
14	1,08	1,07	1,05	1,04
15	1,05	1,05	1,02	1,01
16	1,03	0,99	0,97	0,98
17	0,97	0,94	0,91	0,95
18	0,89	0,89	0,88	0,89
19	0,86	0,85	0,84	0,87
Average	1,21	1,20	1,19	1,20
*sd	0,22	0,23	0,23	0,24
*CV%	18,51	18,84	19,18	19,91
*L	1,69	1,68	1,67	1,70
*S	0,86	0,85	0,84	0,87
*L-S	0,83	0,83	0,83	0,83
*L+S	2,55	2,53	2,51	2,57
*L/S	1,97	1,98	1,99	1,9

*sd - standard deviation, *CV% - coefficient of variation, *L – longest chromosome,
*S – shortest chromosome, *L-S - difference of longest and shortest chromosome,
*L+S – sum of longest and shortest chromosome, *L/S - ratio between longest and shortest chromosome

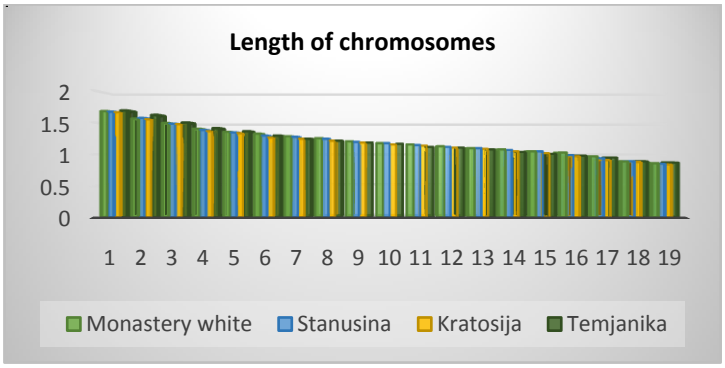


Chart 1. Graphical display of the length of the chromosome

Table 3. Percentage of fecundation in isolated conditions (self-pollination) and in normal conditions(cross-pollination), percentage of alive pollen and percentage of pollen germination

Cultivars	Isolated conditions (autogamy) %	Normal conditions (xenogamy) %	Alive pollen %	Pollen germination %
Monastery white (Klis üzum)	29,95	79,81	89,10	79,18
Stanusina	28,67	77,24	88,71	77,63
Kratosija	24,16	59,15	78,12	69,35
Temjanika	30,80	82,30	87,46	75,40

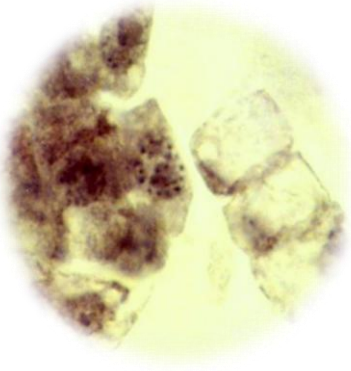


Fig.1 Metaphase chromosomes of grape varieties

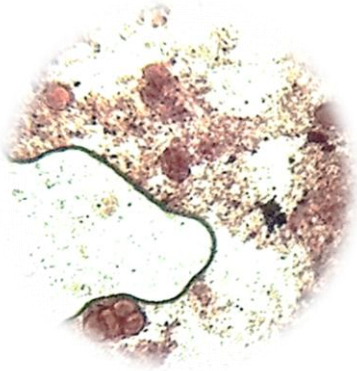


Fig. 2 Meiotic chromosomes of grape varieties

Fig. 3 Monastery white (Klis üzum)

Fig. 4 Temjanika

Fig. 5 Stanusina

Fig. 6 Kratosija

Conclusions

At the examined grape varieties - monastery white (klis üzum), stanusina, kratosija and temjanika according to the cariotype it is stated 2n = 38, that is typical for these cultivars. The chromosomes are very small and they are difficult to be found and observed. The best fertilization showed Temjanika variety, best germination of pollen showed white variety Monastery and lowest fertility slightest germination of pollen showed the variety Kratosija. In the observed phenotype of the examined varieties, that sometimes may drastically reflect the cariotype and the polyploidia, no differences of that kind are noticed. The cell division is regular and the metaphase is normal. According to the existing limited possibilities for examination, abnormalities of the chromosomes

are not noticed (aberrations, divisions etc.). Insignificant abnormalities in fertilization have been shown in the Kratosija variety, because we observed certain abnormalities in the meiotic chromosomes at the fertilization stage in the flower. So sometimes in this variety is less germination of pollen and less fertilization. The smallest length of the chromosome was measured in the Kratosija variety 0.84 µm, and the highest in the Temjanika variety 1.70 µm. Examination of cytogenetic status in varieties contributes to determining the similarity between varieties and belonging to a particular group or subgroup. Also, the cytogenetic status contributes to determining the centers of origin.

References

1. Aradhya, M., Wang, Y., Walker, M.A., Prins, B.H., and col. 2013. Genetic diversity, structure, and patterns of differentiation in the genus *Vitis*. Plant Systematics and Evolution, Vienna, v.299, n.2, p.317-330.
2. Bouquet A: Grapevines and Viticulture. In Genetics, Genomics, and Breeding of Grapes. Edited by Adam-Blondon A-F, Martinez-Zapater JM, Kole C.: Science Publishers; 2011:1-29.
3. M.Vucinic, V. Pesic (1997) Manipulation of animal and plant genomes and genes in agriculture. Ed. Books, University of Belgrade.
4. Peruzzi, L.; Eroglu, H.E. (2013) Karyotype asymmetry: again, how to measure and what toMeasure. Comparative Cytogenetics, St. Petersburg, v.7,n.1, p.1-9.
5. Pierozzi, N.I. (2011), Karyotype and nor-banding of mitotic chromosomes of some *Vitis* L. species. Revista Brasileira de Fruticultura, Jaboticabal, v.E, p. 564-570, Volume Especial.
6. Sharakhova MV, George P, Brusentsova IV, Leman SC, Bailey JA, Smith CD, Sharakhov IV: Genome mapping and characterization of the Anopheles gambiae heterochromatin. BMC Genomics 2010, 11:459.
7. This, P.; Lacombe, T.; Thomas, M.R. 2006. Historical origins and genetic diversity of wine grapes. Trends in Genetics, Cambridge, v.22, n.9, p.511-519.
8. Wolf T. and col. 1999. Reliable identification of grapevine rootstock varieties using RAPD PCR on woody samples. Australian Journal of grape and wine Research 5, 34-38.
9. http://www.biologydiscussion.com
10. http://www.oiv.org
11. https://www.ncbi.nlm.nih.gov
12. http://www.pnas.org
13. http://www.ecpgr.cgiar.org/Databases/Crops /Vitis.htm
14. https://www.ncbi.nlm.nih.gov/books/
15. http://www.vivc.de
16. GWRDC innovators network
17. www.gwrdc.com.au
18. www.statisticshell.com