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### S. GORYSLAVETS <sup>1</sup>, V. RISOVANNA <sup>1</sup>, R. BACILIERI <sup>2</sup>, J.-F. HAUSMAN <sup>3</sup>, M. HEUERTZ <sup>3, 4, 5</sup>

National Institute for Vine and Wine «Magarach»; Ampelography, Breeding and Genetics of Grapevine, Yalta, Ukraine E-mail: goricvet\_2@rambler.ru

<sup>2</sup> Institut National de la Recherche Agronomique, Centre INRA de Montpellier 2 Place Viala, 34000 Montpellier, France

 Centre de Recherche Public-Gabriel Lippmann; Environment and Agro-Biotechnology; rue du Brill 41; L-4422 Belvaux, Luxembourg
 Université Libre de Bruxelles; Evolutionary and Ecology cp160/12; av. F.D. Roosevelt 50; B-1050 Brussels, Belgium

<sup>5</sup> Centre of Forest Research CIFOR-INIA; Forest Systems and Resources; ctra. de la Coruña km 7.5; E-28040 Madrid, Spain

## A PARENTAGE STUDY OF CLOSELY RELATED UKRAINIAN WINE GRAPE VARIETIES USING MICROSATELLITE MARKERS

Four bred grapevine varieties released for commercial cultivation in Ukraine, namely 'Antey Magarachskii', 'Rubinovyi Magaracha', 'Granatovyi Magaracha' and 'Rubin Golodrigi', and their putative parental forms were genotyped using six microsatellite loci. Genotypes were compared with breeding records to verify genetic relationships among varieties. Results of the analysis confirmed four of six parent-offspring relationships. Results of the analysis allow to assume that genotype 'Seyve Villard 20347' is the direct parent of 'Antey Magarachskii' instead of its grandparent. The first-studied accession believed to be that of Granatovyi Magaracha was identified as impurity. In order to verify the parentage of Granatovyi Magaracha, rest accessions of that variety and its putative parent Antey Magarachskii were additionally genotyped at 13 nuclear loci and at three chloroplast loci. The parent-offspring relationship was confirmed, as all Granatovyi Magaracha accessions had a common allele with the parent variety Antey Magarachskii at each locus and the same chlorotype A. Different Granatovyi Magaracha accessions could have been obtained via vegetative propagation of two seedlings which arose from one crossing.

**Introduction.** The south of Ukraine has a longstanding tradition of growing table and wine grape varieties. The latter are made into wines distinguished for their excellent quality. Recently, new wine grapes produced by generative breeding are being introduced into commercial cultivation. This helps improving and enlarging the countryrs wine grape assortment since such varieties have better economical characters. Four newly-bred promising vintage varieties are the focus of this paper: 'Rubin Golodrigi', bred in 1974 and released by the Research Company «Ampelos»; 'Rubinovyi Magaracha', 'Antey Magarachskii', and 'Granatovyi Magaracha' were bred, respectively, in 1928, 1971 and 1982 and released by the National Institute for Vine and Wine «Magarach». These varieties are highly resistant to diseases and can be elaborated into table and dessert wines. They have started to be widely grown in Russia and in Ukraine. 'Rubinovyi Magaracha' and 'Rubin Golodrigi' have juicy fruits with a flavour of the berry of nightshade. 'Antey Magarachskii' has fruits with a crisp juicy flesh distinguished for chocolate flavours. Unlike the above-mentioned grapes, the fruit of 'Granatovyi Magaracha' produces coloured juice, enabling its use as teinturier [1].

An impressive diversity of varieties, forms and species are involved in grapevine breeding. Their origin is sometimes uncertain, which is why their identification and the characterization of variety pedigrees is an important task. The need to identify interspecific grapevine hybrids is especially important for the south of Ukraine where grape and wine growing dates back to Greek colonists of antiquity and breeding activities have been extensive since the middle of the 19th century [2]. Historically, ampelographic methods were the only tool used to this end and relied mostly on visual characters of the leaves, clusters and shoot apices. Unfortunately, the potential and usefulness of this type of identification are restricted due to considerable variation of the characters and subjectivity of human estimates. Recently, microsatellite markers (simple sequence repeats – SSRs) have come into use to investigate genetic diversity of grapevine [3, 4] and to establish genetic relationships among varieties [5–8]. They offer a number of advantages, including high polymorphism and a co-dominant mode of inheritance. They allow a precise molecular fingerprint of grape genotypes and have proved to be the most informative and popular type of DNA

markers as concerns identification of varieties and determination of their parentages [3, 5, 7-11].

The four varieties in the focus of this paper have already been studied for a number of years using conventional ampelographic characters, biochemical indices and several morphometric variables [12–14]. They constitute valuable breeding material and have been used in numerous crossings. 'Antey Magarachskii' is a parent of several new varieties, such as 'Krasen', 'Pamyati Golodrigi', 'Safyanovyi' and of eleven new forms. We report here on genetic fingerprinting of the four varieties and four putative parents of them in order to evaluate their parentages suggested by breeding records.

The research was done at the Centre de Recherche Public — Gabriel Lippmann (Luxembourg) and the National Institute for Vine and Wine «Magarach» (Yalta, Ukraine) in the framework of the international project «Conservation and Sustainable Use of Grapevine Genetic Resources in the Caucasus and in the Northern Black Sea Region» coordinated by Bioversity International (formerly: IPGRI). The accessions of the variety Granatovyi Magaracha and Antey Magarachskii were genotyped at the Institut National de la Recherche Agronomique (INRA, France) in the context of a research programme supported by the National Institute of Agricultural Research of France (called ECO-NET).

Materials and Methods. Plant material and DNA extraction. The plant material used for genetic characterization in this study are four wine grape varieties with black berries, namely 'Antey Magarachskii', 'Rubinovyi Magaracha', 'Granatovyi Magaracha' and 'Rubin Golodrigi'and some of their putative parents, namely the Georgian autochthonous variety 'Saperavi', the old Moldavian variety 'Maiskii Chernyi', the widely grown French variety 'Cabernet Sauvignon' and the Vitis interspecific hybrid Seyve Villard 20347 (Table 1). 'Rubin Golodrigi', 'Antey Magarachskii' and 'Granatovyi Magaracha' possess multiple resistances to pests and diseases [15, 16]. These varieties are of complex interspecific origin since Vitis interspecific hybrids ('Seyve Villard 20347', 'Magarach 6-68-27', 'Magarach 85–64–16', etc.) are reported to have been used as their parents at different stages of the breeding process (Figure). The variety 'Saperavi' is a teinturier with a lot of pigments in its skins,

which encourages its use as a colour-enhancing element in white and red wine technologies [17, 18]. Unfortunately, all putative parents could not be analysed since some of them have been lost. For DNA extraction young shoots without symptoms of pathology were collected from accessions of the test varieties growing in the collection of the Institute «Magarach» (experiment farm in the village of Vilino, Ukraine). The shoots were frozen in liquid nitrogen and stored at a temperature of -86 °C. DNA was extracted from the leaf tissue following the method of Lefort and Roubelakis-Angelakis [19].

In order to standardize genotyping results according to the «European Vitis Database», we additionally analysed 28 varieties [20] for which genotypes were published in This et al. [21]. Cuttings from accessions of these varieties were kindly provided by Dr. Didier Vares from the Institut National de la Recherche Agronomique (INRA), Vassal, France. Buds were dissected out and DNA was extracted using the NucleoSpin Plant kit (Macherey Nagel). Accessions of 'Cabernet Sauvignon' from both the collection of the «Magarach» Institute in Ukraine and from the INRA Vassal collection in France have been included in the analysis.

Microsatellite analysis. We chose microsatellite primers widely used for genetic fingerprinting of grape varieties and recommended by «Bioversity International» and the «European Vitis Database» [21]: VVS2 [22], VVMD5, VVMD7 [23, 24], VVMD27 [24], VrZAG62 and VrZAG79 [25]. PCRs (10 µl) were performed using 0.25 U of FideliTaq polymerase (GE Healthcare), 1X reaction buffer (10 mM Tris-HCl (pH 8.6), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>) (GE Healthcare), 2 mM of each dNTP, 0.4 µM of each primer and 1 µl of DNA extract diluted 40 times than give concentrations. PCR conditions were as follows: 95 °C for 4 min, 40 cycles of 94 °C for 30 sec, 52 °C for 20 sec, 72 °C for 1 min, followed by a final extension for 7 min at 72 °C and cooling to 4 °C.

The forward primer of each microsatellite locus was labelled with a fluorescent dye (6-FAM, PET, NED or VIC, Table 2) to visualize PCR amplification products from all loci in the same analysis run on an automated monocapillary sequencer (ABI Prism 310, Applied Biosystems). Allele sizes were

Table 1
Grape cultivars included in this study and parentage information on them on basis breeding reports

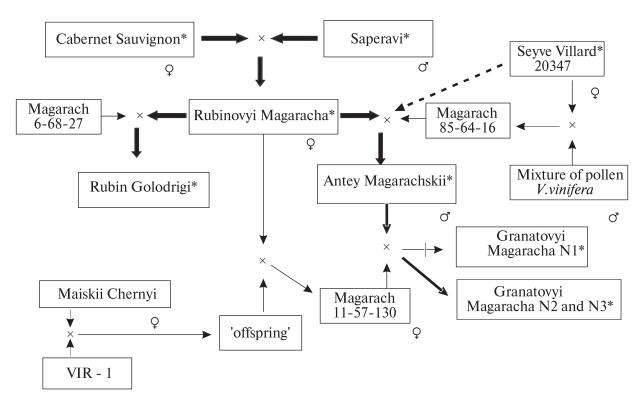
Genus	Species	Variety name	Possible synonyms	Parentage (A × B)	Use (W, Wine grape; T, Table grape)	Breeding station	Authors
Vitis	Hybrid *	Antey Maga- rachskii	Magarach 70-71-52	Rubinovyi Maga- racha × Magarach 85-64-16	T, W	NIVW «Maga-rach» **	P. Golodriga, V. Usatov, L. Troshin, Yu. Mal'chikov, N. Dubovenco
Vitis	Hybrid	Granatovyi Magaracha	Magarach 77-81-3	Antey Magarachskii × × Magarach 11-57-130	W	NIVW «Maga-rach»	V. Usatov, L. Kireeva, P. Golodriga, L. Troshin, V. Volinkin, V. Klimenko, N. Oleynikov, Yu. Mal'chikov
Vitis	vinifera L.	Cabernet Sauvignon		$\begin{array}{c} Cabernet \; franc \times Sau-\\ vignon \; blanc \end{array}$	W	Old French variety	
Vitis	Hybrid	Rubin Golo- drigi	Magarach 15-74-29	Rubinovyi Magara- cha × Magarach 6-68-27	W	Research Company «Ampelos»	P. Golodriga, M. Kostik, V. Yurchenko
Vitis	vinifera L.	Rubinovyi Magaracha	Magarach 56	Cabernet Sauvignon × × Saperavi	W	NIVW «Maga-rach»	N. Paponov, V. Zotov, P. Tsarev, P. Golodriga
Vitis	vinifera L.	Saperavi		Unknown	W	Autochtonous Georgian vari- ety	
Vitis	Interspeci- fic cross	Seyve Villard 20347	Perle Noire	V. vinifera × Seyve Villard 12358	T, W		Seyve Villard
Vitis	vinifera L.	Maiskii Chernyi	German black	Unknown	W	Old Moldavian variety	

<sup>\*</sup> Accessions are qualified as hybrids if they have a complex genetic background, involving some non-*vinifera* varieties. \*\*NIVW: National Institute for Vine and Wine.

Table 2
Allele compositions of eight grape genotypes analysed with 6 microsatellite loci (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79) standardized to allele sizes published in This et al. [21]. Missing data are coded with «0»

Variety	VVS2 dye:6-FAM *, stdized to $n = 123$ **		VVMD5 dye: PET stdized to n = 222		VVMD7 dye:6-FAM stdized to n = 232		VVMD27 dye: NED stdized to $n = 175$		VrZAG62 dye: VIC stdized to n = 174		VrZAG79 dye: NED stdized to n = 238	
Cabernet Sauvignon	139	151	232	240	240	240	175	189	188	194	248	248
Rubinovyi Magaracha	133	151	232	240	240	240	0	0	188	200	248	262
Saperavi	133	145	224	240	240	240	189	192	188	200	244	262
Antey Magarachskii	133	145	226	232	240	250	179	189	186	188	248	256
Granatovyi Magaracha N1	133	145	234	234	240	240	0	0	196	196	244	244
Maiskii Chernyi	143	143	226	234	240	250	179	189	188	194	238	244
Rubin Golodrigi	133	151	232	240	240	252	192	194	194	200	246	248
Seyve Villard 20347	145	149	226	232	250	252	179	189	186	194	256	262

<sup>\*</sup>The fluorescent dyes used to label forward primers of each locus were 6-FAM, PET, NED or VIC. \*\* Allele sizes are expressed in base pairs (bp). \*\*\* «Stdized to n = 123» means that the length of the shortest PCR product discovered at that particular by This et al. [21] was 123 bp; it is the allele size to which PCR fragment lengths in this study were standardized.



Crossing records of the focal varieties and their parental forms. Varieties subjected to genetic analysis are marked with an asterisk (\*). Relationships suggested by genetic fingerprinting are depicted with bold arrows. The dotted arrow represents a new relationship suggested by genetic data. The parent-offspring relationship between 'Antey Magarachskii' and 'Granatovyi Magaracha N1' suggested from the breeding design was not confirmed with genetic data (bold bars)

determined as PCR product lengths by comparison with an internal size standard (GeneScan<sup>TM</sup> 500 LIZ®) using GeneMapper 3.0 software (both Applied Biosystems). They were recorded in base pairs (bp) with a two decimals precision. The comparison of the allele sizes obtained for reference varieties to the This et al. [21] data allowed standardization of allele size of the complete data set in order to achieve compatibility with the format of the European Vitis Database.

In order to verify the parentage of Granatovyi Magaracha, two others accessions of that variety (Granatovyi Magaracha N2 and N3) and its parent Antey Magarachskii were additionally analyzed for 13 nuclear loci and for three chloroplast loci at the Institut National de la Recherche Agro-nomique (INRA, France) on an automated capillary sequencer (ABI Prism 3130X, Applied Biosystems).

Data analysis. Genetic diversity statistics of each locus in the total number of eight target genotypes were computed using the SPAGeDi

version 1.2 software [26]. Diversity was estimated as number of alleles and expected heterozygosity (gene diversity). First degree parentage relationships (parent-offspring relationships) were identified using GIMLET version 1.3.3 [27]. Absolute coefficients of molecular coancestry (kinship) between pairs of varieties were computed as averages over loci according to Lynch and Walsh [28]. The coefficient of coancestry between two varieties is defined as the probability that two randomly drawn genes at a locus, one in each variety, are identical by descent. In our estimation, we assume that genes are identical by descent if they show the same PCR product length in microsatellite analysis, i.e. if they are identical in state. We hence do not account for possible inbreeding of varieties. The reason for this is that the base population of the varieties from which inbreeding coefficients could be computed is unknown.

**Results and Discussion.** Genotypes of eight focal (Table 2) and twenty-eight reference varieties

[20] were obtained at six microsatellite loci. The accession of 'Cabernet Sauvignon' from the collections of the Institute «Magarach» was found to be genetically identical to that of INRA at all six microsatellites. No clear amplification product could be obtained in 'Rubinovyi Magaracha' and 'Granatovyi Magaracha' at locus VVMD27 despite repetitive PCRs. Non-amplification might be due to a mutation in one of the PCR primer annealing sites, causing a «null allele» [29]. The missing data are coded with «0». Genetic diversity statistics of microsatellite loci are summarized in Table 3. The total number of alleles observed at the six loci was 30. The lowest polymorphism was observed at locus VVMD7 with 3 alleles and a level of heterozygosity equal to  $H_E = 0.508$ , the highest polymorphism occurred at VVS2 and VrZAG79 with each 6 alleles and heterozygosity equal to  $H_E =$ = 0.833 (Table 3).

Putative first degree parentage relationships among the focal varieties were identified using GIMLET v1.3.3 software and compared with the available crossing records (Figure). 'Rubinovy Magaracha' was found to be a compatible offspring of a cross between 'Cabernet Sauvignon' and 'Saperavi', sharing 50 % of its allele composition with each of its putative parents. Similarly, the genetic data confirmed that both 'Rubin Golodrigi' and 'Antey Magarachskii' were compatible offspring of 'Rubinovyi Magaracha' used as a female parent, as suggested from the breeding records. Furthermore, 'Antey Magarachskii' was a compatible offspring of the parent pair 'Rubinovyi Magaracha' and 'Seyve Villard 20347'. This

suggests that 'Seyve Villard 20347' could be the direct male parent rather than a grandparent to 'Antey Magarachskii'. 'Antey Magarachskii' is known to be close to its female parental line, especially to the cultivar 'Cabernet Sauvignon', as concerns morphological characteristics of the leaves and clusters and the taste of the berries. On the other hand good resistance to downy mildew, powdery mildew and adaptation to low temperatures is inherited from its male parent, 'Seyve Villard 20347'. Besides, 'Antey Magarachskii' and 'Seyve Villard 20347' both have two uses of the fruit, either for fresh consumption or vine making [17].

The genetic data showed that the accession of 'Granatovyi Magaracha' does not share one allele at each locus with its supposed male parent 'Antey Magarachskii'; as a matter of fact, three loci (VVMD5, VrZAG62 and VrZAG79) do not support a parent-offspring relationship between Table 3

Characterization of microsatellite loci of the eight grape genotypes described in this paper. H<sub>E</sub>: expected heterozygosity or gene diversity

		size	Variance of allele size	
6	0.833	142.0	49.6	
5	0.817	232.8	28.7	
3	0.508	243.4	27	
5	0.788	186.3	40.8	
5	0.825	192.5	25.9	
6	0.833	249.9	55.2	
	5 3 5 5	5 0.817 3 0.508 5 0.788 5 0.825	6 0.833 142.0 5 0.817 232.8 3 0.508 243.4 5 0.788 186.3 5 0.825 192.5	

Table 4
Matrix of coancestry coefficients between pairs of varieties. In the absence of inbreeding of the parents,
a coancestry coefficient of 0.25 is expected between parent and offspring. Overall high coancestry coefficients
highlight the high relatedness of varieties

Variety	Cabernet Sauvignon	Saperavi	Rubinovyi Magaracha	Antey Magarachskii	Granatovyi Magaracha	Rubin Golodrigi	Seyve Villard	Maiskii Chernyi
Cabernet Sauvignon	0.667							
Saperavi	0.292	0.583						
Rubinovyi Magaracha	0.500	0.450	0.600					
Antey Magarachskii	0.292	0.250	0.300	0.500				
Granatovyi Magaracha	0.200	0.400	0.250	0.200	0.900			
Rubin Golodrigi	0.333	0.250	0.400	0.167	0.150	0.500		
Seyve Villard 20347	0.125	0.125	0.100	0.333	0.050	0.125	0.500	
Maiskii Chernyi	0.208	0.208	0.150	0.250	0.300	0.083	0.208	0.583

Allele compositions of the parent variety Antey Magarachskii and two asseccions of Granatoviy Magaracha analysed with 13 nuclear loci (VVIn16, VIp60, VVIv67, VVMD7, VVMD21, VMC4f3, VVIb01, VVMD28, VVIq52, VVIv37, VVS2, VrZAG62 and VrZAG79) and for three chloroplast microsatellite loci (CCMP3, CCMP5 and CCMP10)

Accession name	VVIn16	VVIp60	VVIq52	VVI	v37	VVIve	67	VMD21	VMC4f3	VVIb01	VVMD28
Antey Magarachskii	147 151	315 315	79 79	149		334 3		247 247		290 294	235 235
Granatovyi Magaracha N2 Granatovyi Magaracha N3		315 319 315 319	79 79 79 79	149 149	,	334 3 334 3		<ul><li>247 247</li><li>247 247</li></ul>		294 294 290 294	235 235 235 245
Accession name	VVMD7	VVS2	VrZA	.G62	VrZ	AG79	CC	2MP3	CCMP5	CCMP10	Haplotip
Antey Magarachskii	240 250	133 145	186	188	248	3 256	1	07	104	115	D
Granatovyi Magaracha N2 Granatovyi Magaracha N3	240 244 240 244	133 133 133 133				256 256		06	105 105	114 114	A A

these varieties. Contrary to other varieties which were heterozygous at four loci at least, 'Granatovyi Magaracha' was heterozygous only for VVS2 and homozygous for VVMD5, VVMD7, VrZAG62 and VrZAG79. This variety showed therefore the highest coefficient of coancestry with itself (0.90, Table 4). The coancestry coefficient technically corresponds to the inbreeding coefficient of the variety's selfed offspring. Moreover, 'Granatovyi Magaracha' also displayed the lowest adaptive variation when morphogenetic responses of the test varieties were studied in vitro [13]. A possible reason for this may be that the variety results from a multi-step breeding process including self-fertilisation.

However, a low degree of allele diversity at neutral loci such as microsatellites is not necessarily associated with a low level of adaptive variation and may be merely coincidental. 'Granatovyi Magaracha' contained alleles that were absent from other genotypes of its putative paternal line (Table 2), namely n + 12 (234) at VVMD5, n + 22(196) at VrZAG62 and n + 6 (244) at VrZAG79. Two of these alleles (234 at VVMD5 and 244 at VrZAG79) were detected in 'Maiskii Chernyi', a putative maternal great-grandparent of 'Granatovyi Magaracha', indicating that they could have been inherited from the putative female line. Unfortunately, the hybrid form 'Magarach 11-57-130' which is the supposed female parent of 'Granatovyi Magaracha' has been lost, so that it has become impossible to verify this suggestion. Our results indicate that the origin of 'Granatovyi Magaracha' is so far not clear and needs further investigation. In this connection, the remaining accessions of the variety Granatovyi Magaracha (N2 and N3) growing in the collection of the Institute for Vine and Wine «Magarach» were genotyped at the Institut National de la Recherche Agronomique (INRA, France).

Their relationships with each other and the parent-offspring relationship with the putative parent variety Antey Magarachskii were analyzed for 13 nuclear loci (VVIn16, VIp60, VMC4f3, VVIb01, VVMD7, VVMD21, VVMD28, VVIq52, VVIv37, VVS2, VrZAG62 and VrZAG79) and for three chloroplast microsatellite loci (CCMP3, CCMP5 and CCMP10). As a result, it was found that the genotypes of the two accessions were identical to that of Antey Magarachskii at loci VVMD21, VVIq52 and VVIv37 while each accession shared one common allele with Antev Magarachskii at the remaining ten loci (Table 5). Thus, the percentage of shared alleles in the genotypes of the two accessions of Granatovyi Magaracha (0.7) indicates their close relatedness. They could have been obtained via vegetative propagation of two seedlings which arose from one crossing.

This is compatible with results of analysis of the accessions for chloroplast microsatellite loci CCMP3, CCMP5 and CCMP10. Chlorotype D

(107/104/115) was identified in the parent form Antey Magarachskii.

The two accessions of Granatovyi Magaracha had the same chlorotype A (106/105/114), which suggest that they descend from one female form as chloroplast inheritance is matrilinear. Thus, analysis of the accessions of Granatovyi Magaracha confirmed the parent-offspring relationship of the male parent Antey Magarachskii and the former variety.

Overall, fairly high coefficients of coancestry were detected between the varieties investigated (Table 4), considering that many values are higher than 0.25, which is the average expectation for a parent-offspring relationship ignoring inbreeding [28]. However, despite the overall high relatedness, each variety was found to have a unique SSR profile.

The results from this study highlight the usefulness of microsatellites in parentage analysis and for verifying pedigree information in grapevine, as has been observed by other authors [6-8].

Genetic data in our study were compatible with five out of six parent-offspring relationships tested (Figure), and they all included 'Rubinovyi Magaracha'. However, to safely confirm these relationships, data at 30 to 50 micro- satellites would be necessary [24]. Genetic data allowed to identify first-studied accession of Granatovyi Magaracha as impurity and to suggest 'Seyve Villard 20347' as the father of 'Antey Magarachskii'.

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# БЛИЗКОРОДСТВЕННЫХ УКРАИНСКИХ ТЕХНИЧЕСКИХ СОРТОВ ВИНОГРАДА С ИСПОЛЬЗОВАНИЕМ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ

Четыре селекционных сорта винограда Антей Магарачский, Рубиновый Магарача, Рубин Голодриги и Гранатовый Магарача, которые культивируются в Украине для приготовления сухих и крепленых вин, и их предполагаемые родительские формы были генотипированы с использованием шести микросателлитных локусов. Для оценки генетических взаимоотношений полученные генотипы были проанализированы на соответствие их селекционной схеме. Результаты анализа подтвердили четыре связи родитель-потомок из шести. Результаты проведенного анализа позволяют предположить, что генотип Сейв Виллард 20347 является прямым родителем Антея Магарачского, а не его прародителем. Проанализированный образец сорта Гранатовый Магарача не соответствовал отношению родитель-потомок и был идентифицирован как примесь. Чтобы уточнить происхождение упомянутого сорта, остальные два образца были дополнительно проанализированы по 13 ядерным и трем хлоропластным локусам. Анализ наследования показал, что изученные образцы имели общий материнский хлоротип, а в каждом из 13 локусов имели общий аллель с отцовским сортом Антей Магарачский, что соответствует генотипам сеянцев от одного скрещивания.

> С. Гориславець, В.Рісованна, R. Bacilieri, J.-F. Hausman, M. Heuertz

### ВИВЧЕННЯ РОДОВОДУ БЛИЗЬКОСПОРІДНЕНИХ УКРАЇНСЬКИХ ТЕХНІЧНИХ СОРТІВ ВИНОГРАДУ З ВИКОРИСТАННЯМ МІКРОСАТЕЛІТНИХ МАРКЕРІВ

Чотири селекційних сорти винограду Антей Магарацький, Рубіновий Магарача, Рубін Голодриги та Гранатовий Магарача, що культивують в Україні для приготування сухих і десертних вин, та їх потенційні батьківські форми були генотиповані з використанням шести мікросателітних локусів. Для оцінки генетичних взаємовідносин отримані генотипи були проаналізовані на їх відповідність селекційній схемі. Результати аналізу підтвердили чотири зв'язки батько — нащадок із шести. Результати проведеного аналізу дозволяють припустити, що генотип Сейв Віллард 20347 є прямий батько Антея Магарацького, а не його прабатько. Проаналізований зразок сорту Гранатовий Ма-

гарача не відповідав відношенню батько — нащадок і був ідентифікований як домішка. Для уточнення походження Гранатовий Магарача решта зразків була додатково проаналізована за 13 ядерними і трьома хлоропластними локусами. Аналіз спадкування показав, що вивчені зразки мали материнський хлоротип, а в кожному із 13 локусів мали спільний алель із батьківським сортом Антей Магарацький, що відповідає генотипам сіянців від одного схрещування.

#### REFERENCES

- 1. *Трошин Л.П.* Производственные сорта винограда Евразии. Краснодар : Кубан. гос. аграр. ун-т, 2006. 208 с.
- Carter J., Pashkevich G. Cultivated plants of the northern Pontos during the Greek colonization // Development of National Programmes on Plant genetic Resources in Southeastern Europe: Proc. Second project meeting. Yalta, 2004. P. 48—49.
- 3. Sefc K.M., Lopes M.S., Lefort F. et al. Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars // Theor. and Appl. Genet. 2000. 100. P. 498–505.
- 4. Aradhya M.K., Dangl G.S., Prins B.H., Boursiquot J.-M,. Walker M.A., Meredith C.P., Simon C.J., Genetic structure and differentiation in cultivated grape, Vitis vinifera L. // Genet. Res. Cambridge. 2003. 81. P. 179—192.
- Sefc K. M., Steinkellner H., Glössl J., Kampfer S., Regner F. Reconstruction of a grapevine pedigree by microsatellite analysis // Theor. and Appl. Genet. 1998. 97. P. 227–231.
- Bowers J.E., Boursiquot J.M., This P., Chu K., Johansson H., Meredith C. Historical genetics: the parentage of Chardonnay, Gamay and other wine grapes of northeastern France // Science. 1999. 285. P. 1562–1565.
- 7. *Vouillamoz J., Maigre D., Meredith C. P.* Microsatellite analysis of ancient alpine grape cultivars: pedigree reconstruction of *Vitis vinifera* L. Cornalin du Valais // Theor. and Appl. Genet. 2003. **107**. P. 448—454.
- 8. *Vouillamoz J.F., Maigre D., Meredith C.P.* Identity and parentage of two alpine grape cultivars from Switzerland (*Vitis vinifera* L. Lafnetscha and Himbertscha) // Vitis. 2004. **43**. P. 81–87.
- 9. Faria M.A., Magalhaes R., Ferreira M.A., Meredith C.P., Ferreira Monteiro F. Vitis vinifera must varietal authentication using microsatellite DNA analysis (SSR) // J. Agricult. and Food Chem. 2000. 48. P. 1096—1100.
- 10. Lefort F., Massa M., Goryslavets S., Risovanna V., Troshin L. Genetic profiling of Moldavian, Crimean

- and Russian cultivars of *Vitis vinifera* L. with nuclear microsatellite markers // Oenologie : 7e symp. intern. d'oenologie. Paris, 2003. P. 71–73.
- Martinez L.E., Cavagnaro P.F., Masuelli R.W., Zuniga M. SSR-based assessment of genetic diversity in South American Vitis vinifera varieties // Plant Sci. – 2006. – 170. – P. 1036–1044.
- Гориславец С.М. Индуцирование ризогенеза экзогенными фитогормонами у винограда in vitro// Тр. Науч. центра виноградарства и виноделия. — Ялта, 2000. — Т. 2, кн.2.— С. 26—29.
- Гориславец С., Рисованная В. Влияния 2,4-Д, НУК, БАП на индукцию ризогенеза и каллусообразования в первичном каллусе двух сортов винограда // Бюл. Гос. Никит. бот. сада. — 2004. — Вып. 89. — С. 44—47.
- 14. Гориславец С., Рисованная В., Сказан О. Антоциановые профили в каллусной культуре и ягоде окрашенных сортов винограда // Современные достижения биотехнологии в виноградарстве и других отраслях сельского хозяйства: Материалы конф. Новочеркасск, 2005. С. 216—225.
- Ампелография СССР. Отечественные сорта винограда / Под ред. П.Я. Голодриги. М.: Легкая промышленность, 1984. 503 с.
- Костик М.А., Юрченко В.Ю. Виноград XXI века, новые сорта Украины // Виноделие и виноградарство. – 2005. – 2. – С. 38–39.
- 17. *Ампелография* СССР / Под ред. А.М. Фролова-Багреева. М.: Пищепромиздат, 1954. 3. С. 66–95.
- Ампелография СССР / Под ред. А.М. Фролова-Багреева. – М.: Пищепромиздат, 1955. – 5. – С. 203– 230
- 19. Lefort F., Roubelakis-Angelakis K.A. Genetic comparison of Greek cultivars of Vitis vinifera L. by nuclear microsatellite profiling // Amer. J. Enol. and Viticult. 2001. 52. P. 101—108.
- 20. Heuertz M., Goryslavets S., Hausman J.F., Risovanna V. Characterization of grapevine accessions from Ukraine using microsatellite markers // Amer. J. Enol. and Viticult. 2008. 59. P. 169–178.
- 21. *This P., Jung A., Boccacci P. et al.* Development of a standard set of microsatellite reference alleles for identification of grape cultivars // Theor. and Appl. Genet. 2004. **109**. P. 1448—1458.
- 22. *Thomas M.R., Scott N.S.* Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs)// Theor. and Appl. Genet. 1993. **86**. P. 985–990.
- 23. Bowers J.E., Dangl G.S., Vignani R., Meredith C.P. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (Vitis vinifera L.) // Genome. 1996. 39. P. 628–633.
- 24. Bowers J.E., Dangl G.S., Meredith C.P. Development

- and characterization of additional microsatellite DNA markers for grape // Amer. J. Enol. and Viticult. 1999. 50. P. 243-246.
- 25. Sefc K.M., Regner F., Turetschek E., Gl?ssl J. Steinkellner H. Identification of microsatellite sequences in Vitis riparia and their applicability for genotyping of different Vitis species // Genome. 1999. 42. P. 367–373.
- 26. *Hardy O.J.*, *Vekemans X. Spagedi*: a versatile compute program to analyse spatial genetic structure at the individual or population levels // Mol. Ecol. Notes. 2002. 2. P. 618–620.
- 27. *Valière N. Gimlet*: a computer program for analysing genetic individual identification data// Mol. Ecol. Notes. 2002. 2. P. 377–379.
- Lynch M., Walsh B. Genetics and Analysis of Quantitative Traits. – Sunderland: Sinauer Assoc., Inc., 1998.
- 29. *Pemberton J.M., Slate J., Bancroft D.R., Barrett J.A.*Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies // Mol. Ecol. 1995. 4. P. 249–252.

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