MICROSATELLITE CHARACTERISTICS OF GRAPEVINE CULTIVARS INCLUDED TO UKRAINIAN STATE REGISTER OF PLANT VARIETIES.

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Thirteen wine and mixed type of use grapevine cultivars from Ukrainian State Register of Plant Varieties, were analyzed at 9 microsatellite loci: VVS2, VVMD5, VVMD7, VVMD27, VVMD25, VVMD28, VVMD32, VrZAG62 and VrZAG79. The number of alleles observed per locus ranged from 4 to 12 and observed heterozygosity values ranged from 0.846 to 1.00. The dendrogram which reflects genetic distances between varieties were obtained by cluster analysis based on the UPIGMA algorithm.

***Key words**: grapevine, microsatellite, SSR, Vitis vinifera L, cultivar identification.*

Due to the significant genetic variability of *Vitis vinifera* L. (approximately 5000 varieties exist today [1]), the microsatellite analysis for their identification is actual.

Grapevine genetic pool of National Scientific Center "Tairov Research Institute of Viticulture and Wine-Making" (NSC) consists of approximately 11 000 plants: 10 000 seedlings of different cross combinations and 700 cultivars. Some of these cultivars are included to State Register of Plant Varieties suitable for dissemination in Ukraine [2]. NSC's grapevine cultivars collection represents the results of breeding process during a large period of time in many countries. Many cultivar synonyms exist and closely related varieties have similar phenotypes, so the clear identification of cultivars is required for successful breeding process [1]. Due to their high level of polymorphism, co-dominance and reproducibility microsatellites were chosen by researchers as the

most reliable tools for cultivar identification and evaluation of genetic diversity [3, 4].

Some investigations in the field of grapevine collection cultivars microsatellite profiling were conducting in NSC's since 2008 [6-8]. In our work we continued these investigations by expanding of marker panel to standard set of nine microsatellites [] and inclusion of not studied cultivars.

The aim of our investigation was the identification of grapevine cultivars included to Ukrainian State Register of Plant Varieties by using a set of SSR markers.

Materials and methods. In this study thirteen cultivars (таb. 1) which was obtained from the Tairov Research Institute of Viticulture and Wine-Making grapevine collection were analyzed. Two reference varieties ("Cabernet Sauvignon" and "Chardonnay") presented in the collection were also included in the analysis to compare allele size results with those of other laboratories. Villard Blan allele characteristics used to confirm the origin of cultivar Golubok were obtained [16] only for 6 loci and are shown in Tab. 1 unchanged.

1. The parentage of studied cultivars.

Variety	**Type of use**	**Parentage***
Golubok	wine	Severnyj x 40 let Oktiabria [8]
Zagrey	wine	Aligote x Ovidiopolskii
Ovidiopolskii	wine	Severnyi x Odeskii ustoichivii
Odesskii chernyi	wine	Alicante Henri Bouschet x Cabernet Sauvignon
Rodnichok	wine	Villard Blan x Ilichvskii rannii (Severnyi x Odesskii ustoichivyi)
Rubin tairovskii	wine	Odeskii ustoichivii x Varousset
Muskat odesskii	wine	Muskat chernyi rannii x Pierrelle (Villard blan x Panse)
Aromatny	table/wine	Vertes csillaga (Eger 1 x Medok noir) x Romulus

Dnestrovskii rozovyi	table/wine	VIRa II 35-20 (Nimrang x Amoursky) x Mathiasz Janos
Zolotistyi ustoichivyi	table/wine	VIRa II 35-20 (Nimrang x Amoursky) x ? (free pollination)
Kometa	table/wine	Tair x Burevestnik

* Data are available in the Vitis International Variety Catalogue (VIVC) [9].

Plant material and DNA extraction. Total genomic DNA was extracted from young leaves frozen to below –20 ºC by using a Qiagen DNeasy Plant Mini Kit according to the instructions provided by the manufacturer. The quantity and quality of purified total DNA were evaluated by electrophoresis on 0.8% agarose gel.

Microsatellite analysis. Nine SSR primer pairs including VVS2 [4], VVMD5, VVMD7, VVMD27 [10], VVMD25, VVMD28, VVMD32 [11] and VrZAG62, VrZAG79 [12] were chosen. PCR reaction was performed in GeneAmp PCR System 9700 (Applied Biosystem) in 20 μl reaction mixture containing 50 ng DNA, 1 μM of each primer, 100 μM of each dNTPs, 1.5 mM $MgCl_2$, 1U of Taq polymerase and applied PCR buffer. The alleles were detected on a DNA analyzer ABI Prizm 310 (Applied Biosystems) by the program GENE MAPPER 4.0. To obtain reliable results each sample was amplified and analyzed twice at each marker.

Data analysis. The genetic analysis program IDENTITY 1.0 [13] was used for the calculation of number of alleles, allele frequency, expected and observed heterozygosity. The phenogram of genetic distance between analyzed genotypes was constructed with MEGA 4 by using the UPGMA (Unweighted Pair Group Method Arithmetic Average) clustering method [14, 15].

Results and Discussion Identity. We screened 13 grape cultivars using 9 SSR markers. All varieties (except reference cultivars) were created at Tairov National Research Center and included to Ukrainian State Register of Plant Varieties. The investigation has demonstrated that all cultivars have unique

allelic profiles and a high genetic diversity. The specific allele sizes that have been revealed are presented in Tab. 2.

A total of 68 alleles were detected at these 9 SSR loci, with an average allele number per locus 7.56 (Tab. 3). The most informative loci were VVMD28 and VVMD5 with nine alleles while VVMD25 with five alleles was found to be the least informative locus.

The expected heterozygosity (He) ranged from 0,746 at locus VVMD25 to 0.861 at loci VrZAG79 and VVS2 with a mean value of 0.802. The observed heterozygosity (Ho) varied between 0.769 at loci VVMD7 and VVMD32 and 1.0 at loci VVS2, VVMD5, VVMD27. In this study, the values of observed heterozygosity at 3 loci (VVS2, VVMD5 and VVMD27) were higher than the expected heterozygosity values as well as data obtained by [].

In the present study, the average value of observed heterozygosity (0.889) was higher than that obtained for the *V. vinifera* by [ibanez 2003] - 0.707, [4И] – 0.809, [6 И] – 0.68 and similar to results [1И].

2. Allele sizes (bp) of 13 grape cultivars at 9 SSR loci. Cabernet Sauvignon and Chardonnay were used as reference.

№	Cultivar	VVS 2		ZAG 62		VVMD 7		VVMD 27		VVMD 5		VVMD 25		VVMD 28		ZAG 79		VVMD 32	
1	Golubok	131	135	186	190	241	243	178	180	239	249	240	242	250	258	258	262	242	274
2	Zagrey	127	139	196	206	241	249	176	180	231	239	242	252	234	250	246	258	240	274
3	Ovidiopolskii	127	131	190	206	245	249	178	180	239	241	252	258	240	250	258	260	240	252
4	Odesskii chernyi	135	153	190	190	241	245	186	191	229	235	244	252	242	250	250	258	242	274
5	Rodnichok	137	153	186	190	241	243	178	191	239	247	252	258	250	266	248	250	252	252
6	Rubin tairovskii	127	147	190	190	245	245	186	191	235	241	252	258	240	240	248	264	258	274
7	Muskat odesskii	135	145	202	206	249	251	182	186	-	239	244	258	240	272	254	264	240	276
8	Aromatny	127	135	196	206	249	255	182	186	231	241	244	244	250	252	250	264	242	242
9	Dnestrovskii rozovyi	135	153	196	198	241	249	178	186	231	237	252	252	250	272	260	262	236	242
10	Zolotistyi ustoichivyi	137	153	198	206	249	249	178	186	231	237	252	258	224	250	258	260	236	274
11	Kometa	137	139	190	204	237	249	176	182	231	239	244	258	250	250	254	262	252	274
12	Chardonnay	139	145	190	198	241	245	178	186	237	241	242	258	224	234	246	248	242	274
13	Cabernet Sauvignon	141	153	190	196	241	241	172	186	235	243	242	252	240	242	250	250	242	242
	Villard Blan*	135	145	182	196	239	253	178	186	235	239	-	-	-	-	258	274	-	-

* Alleles sizes data for Villard Blan (Seyve Villard 12375) were published previously [16].

3. SSR loci, number of allele (Na), alleles sizes, expected heterozygosity (He), observed heterozygosity (Ho) for 13 grape cultivars analyzed at 9 SSR markers.

Locus	Na	Allele size (pb)	He	Ho
VVS2	9	127, 131, 135, 137, 139, 141, 145, 147, 153	0,861	1
VrZAG62	7	186, 190, 196, 198, 202, 204, 206	0,769	0,846
VVMD7	7	237, 241, 243, 245, 249, 251, 255	0,763	0,769
VVMD27	7	172, 176, 178, 180, 182, 186, 191	0,804	1
VVMD5	9	229, 231, 235, 237, 239, 241, 243, 247, 249	0,842	1
VVMD25	5	240, 242, 244, 252, 258	0,746	0,846
VVMD28	9	224, 234, 240, 242, 250, 252, 258, 266, 272	0,787	0,846
VrZAG79	8	246, 248, 250, 254, 258, 260, 262, 264	0,861	0,923
VVMD32	7	236, 240, 242, 252, 258, 274, 276	0,787	0,769
Total:	68			
Average:	7,5556		0,8022	0,889

The most frequent alleles per locus were VVS2 – 135 and 153 (frequency was 0.192), VrZAG62 – 190 (0.385), VVMD5 – 239 (0.240), VVMD7 – 239 and 249 (0.308), VVMD25 – 252 (0.346), VVMD27 – 186 (0.308), VVMD28 – 250 (0.385), VrZAG79 – 250 and 258 (0.192), VVMD32 – 242 (0.308).

Genetic relationships. The DNA typing information gives an opportunity to determine possible genetic relatedness of the cultivars and, in some cases [17], to disprove declared “parent-progeny” relationships. The using UPGMA method

in cluster analyses provides a better consistency between groups and genealogy [18].

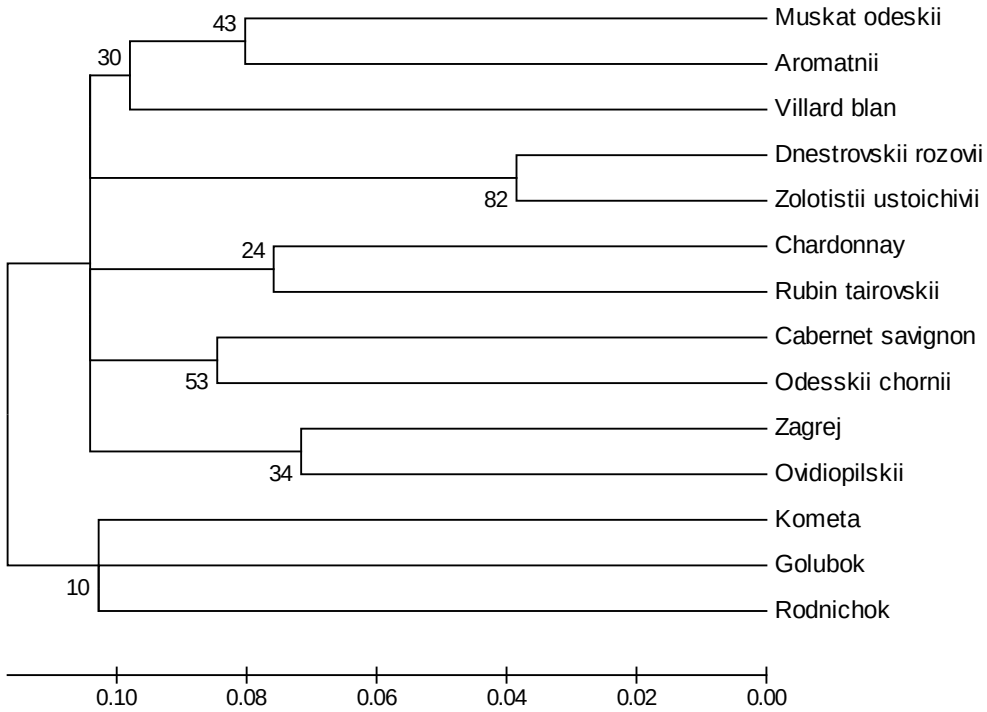

Fig. 1: Dendrogram of genetic distances obtained according to UPGMA clasterization have been applied of microsatellite data analysis bootstrap test

In this work for some cultivars obtained microsatellite profiles were in agreement with their parents genotypes which were presented in investigated sample. Thus Odeskii chernyi and Cabernet Sauvignon, Zagrey and Ovidiopolskii formed separate subclusters on dendrogram.

Due to what parents of investigated cultivars Aromatnii and Muscat Odesskii originated from Villard Blan, they are formed common cubclaster with this cultivar.

Cultivar Rodnichok demonstrated common alleles only with one of his assumed parents – Ilichevskii rannii and was differed from Villard Blan genotype in 5 from 6 studied loci. Because of insignificant similarity between Rodnichok and Villard Blan genotypes they have demonstrated the highest Nei distance on dendrogram. The parentage of cultivar Rodnichok will be investigated more thoroughly further.

Varieties Dnestrovskij rozovyj and Zolotistyj ustoichivyj clustered together because have a common parent – VIRa II 35-20 (Nimrang x *V. amurensis*).

Also on dendrogramme the cultivars Golubok, Rodnichok and Kometa demonstrated the genotype similarity by grouping in one subcluster since they are descendants of variety Severnii, which includes *V. amusensis* in genome. At the same time cultivar Kometa is a distant ancestor of cultivar Severnii and was obtained as result of crossing Tair (Coarna neagra x Dattier de st. Vallier) x Burevestnik (Ferdinand de Lesseps (Chasselas blanc x Isabella) x mixture of pollen Illiskii + Dekorativnyi + Fioletovyi rannii).

Obtained data will be use to study genetic relationships between cultivars and evaluation genetic diversity of Tairov Research Institute grapevine collection.

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МІКРОСАТЕЛІТНІ ХАРАКТЕРИСТКИ СОРТІВ ВИНОГРАДУ, ВКЛЮЧЕНИХ ДО ДЕРЖАВНОГО РЕЄСТРУ СОРТІВ РОСЛИН УКРАЇНИ

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Тринадцять сортів винограду технічного та змішаного напряму використання, що включені до Українського державного Реєстру сортів рослин, були проаналізовані за 9 мікросателітними локусами: : VVS2, VVMD5, VVMD7, VVMD27, VVMD25, VVMD28, VVMD32, VrZAG62 та VrZAG79. Число алелів у локусі коливалось від 4 до 12, а спостережувана гетерозиготність варіювала від 0,846 до 1,00. За допомогою кластерного аналізу за алгоритмом UPIGMA була побудована дендрограма, що демонструє генетичні дистанції між сортами.

Ключові слова: *виноград, Vitis vinifera L, мікросателіт, SSR, ідентифікація.*

МИКРОСАТЕЛЛИТНЫЕ ХАРАКТЕРИСТИКИ СОРТОВ ВИНОГРАДА, ВКЛЮЧЕННЫХ В ГОСУДАРСТВЕННЫЙ РЕЕСТР СОРТОВ РАСТЕНИЙ УКРАИНЫ.

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Тринадцать сортов винограда технического и смешанного направления использования, включенные в Украинский государственный Реестр сортов растений, были проанализированы по 9 микросателлитным локусам: VVS2, VVMD5, VVMD7, VVMD27, VVMD25, VVMD28, VVMD32, VrZAG62 и VrZAG79. Число аллелей в локусе колебалось от 4 до 12, а наблюдаемая гетерозиготность варьировала от 0,846 до 1,00. С помощью кластерного анализа по алгоритму UPIGMA была построена дендрограмма, отражающая генетические дистанции между сортами.

***Ключевые слова**: виноград, Vitis vinifera L., микросателлит, SSR, идентификация.*