Vitis **54** (Special Issue), 37–41 (2015)

Genetic diversity of ancient grape cultivars of the Crimea region

S. GORYSLAVETS[1)], R. BACILIERI[2)], V. RISOVANNAYA[1)], E. MEMETOVA[1)] and V. LAUCOU[2)]

[1)] Laboratory of Molecular-Genetic Research, National Institute for Vine and Wine "Magarach", Yalta, Crimea
[2)] UMR AGAP Amélioration Génétique et Adaptation des Plantes (UMR 1334), INRA - CIRAD - SupAgro, Montpellier, France

Summary

A total of 76 accessions of Crimean autochthonous grape cultivars from the collection of the National Institute of Vine and Wine "Magarach" were genotyped using 22 nuclear and 3 chloroplast microsatellite loci (SSR) to characterize their genetic diversity. The total number of alleles was 238, the mean number of alleles per locus was 10.8 and the range of expected heterozygosity was 0.4-0.88. Several synonyms were identified based on the comparison of microsatellite profiles with INRA and EVD databases. Morphological, eno-carpological and eno-chemical characterization of number of cultivars using BBCH scale and OIV descriptors were performed. A short synopsis of the origin and historical development of Crimean autochthonous grape cultivars is presented.

K e y w o r d s : *Vitis vinifera*; autochthonous cultivars; microsatellites; Crimea; genetic diversity.

Crimea is a peninsula lying between latitudes 40° and 50° North and surrounded by Azov Sea and the Black Sea. The latitudes of Crimea are those of southern France, Italy and Ciscaucasia (Fig. 1). Crimea's landscape is very diverse, with steppes in the North and rock massifs in the South which protect the southern coast from cold air flows. A sharp contrast between the moderate steppe climate of the plain and a nearly submediterranean type of the south coast can be seen on Crimea territory.

The first mentioning of grape growing in Crimea predate the 10th century BC. Different peoples, including Scythians, Greeks, Romans, Goths, Huns, Byzantines, Kazaks, Genoeses and Tatars, populated the region due to its advantageous geographical position, at routes crossroads

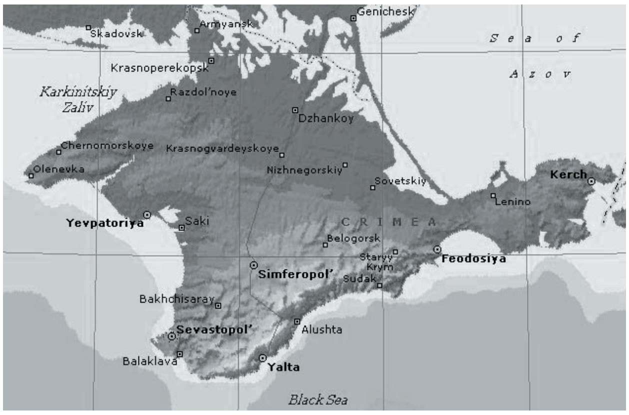

Fig. 1: Map of the Crimean Peninsula.

from Asia to Europe (KORZHINSKII 1904, MALIKOV 1968, NEGRUL 1960, KHRAPUNOV 2010). Ancient Greek colonists who came to the southern coast of Crimea in the 7th-6th centuries BC made a considerable impact on the region's grape growing and wine making. They carried on a brisk trade with the local Scythian tribes populating the steppe of the peninsula and of Ukraine as well as with Greece and Asia Minor. The Silk Road also passed through Crimea. This favored the development of Arts and importations of new grape varieties in the region (NEGRUL 1946, 1960; CARTER *et al.* 2004). In the beginning of our era, the ancient Greek colonies fell under the Roman sway that, after several centuries, gave way to the Byzantine domination. The 13th century saw Mongol Tatars invasion. In that time and onward, another influence on grape and wine growing of the southern coast of Crimea was exerted by Genoese traders. They might have also imported varieties as they did a lively trade in wine. Grape growing flourished best in that time, with Sudak as center themselves.

Ottomans established in Crimea in 1475, followed by a decline in wine making due to the Islamic prohibition. Mostly table grapes continued to be cultivated. The revival of wine making in Crimea begins in the 18th century after Crimea joined the Russian Empire.

The first grapevine collection was established in the Crimea in the Nikita Botanical Gardens founded in 1812 near Yalta. A further enhancement of grape and wine growing was associated with the school of wine making established in 1828 on the location named Magarach. The modern grapevine collection of the National Institute for Vine and Wine "Magarach" maintains more than 3,000 accessions, including about 80 local grape cultivars, and their diversity is of special interest.

Over the 18th-20th centuries, autochthonous grape cultivars of the Crimea were the subject of much investigation (KORZHINSKII 1905, NEGRUL 1958). This subject became part of scientific interests of the Magarach Institute in 1932, and a study of autochthonous grape cultivars was initiated by A.A. Ivanov in the Sudak area where the majority of grapes were located. A considerable contribution to this research was also made by Lazarevskii, Paponov, Gramotenko, Panarina, Troshin, etc. who were with the Institute in different time periods (IVANOV 1947, GRAMOTENKO 1979, DASHKEVICH 1966).

Traditionally, germplasm identification of grape varieties is done based on historical information and the ampelography of an acquisition. The advent of microsatellite markers (SSR) has enabled grape genotypes to be studied at a principally new level unaffected by subjective evaluation and phenotypic variation. This makes them a convenient

Correspondence to: Dr. S. GORYSLAVETS, Laboratory of Molecular-Genetic Research, National Institute for Vine and Wine "Magarach" (Yalta, Crimea). E-mail: goricvet2@gmail.com

instrument to be used with the views to evaluate the origin and the genotypic variation of cultivars, to identify grape genotypes and to reveal synonyms and homonyms (Sefc *et al*. 2000, Labra *et al.* 2002, This *et al*. 2004, Gismondi *et al.* 2014). Earlier we studied a number of autochthonous grape cultivars of the Crimea as part of International Projects (Lefort *et al.* 2003, Heuerts *et al.* 2008). We continued characterization of our cultivars using 22 SSR-markers and OIV descriptors under the EU program COST Action FA1003 and the RFBR grant №15-29-02715. This made it possible to standardize the results obtained in accordance with the recommendations elaborated by EU programs and to compare grape genotypes with those found in the Eu-Vitis (European Vitis Database) and French INRA databases which include more than 7,000 accessions.

This paper reports results arising from our attempts to identify old grape cultivars found in Crimea and to perform for some of them morphological and chemical characterization and to analyze their origin and genetic diversity.

Material and Methods

Grape plant samples: Samples of plant material (leaves, cuttings) of 76 accessions of the Crimean autochthonous grape cultivars were collected from the ampelographic collection of the National Institute of Wine and Vine "Magarach". The accession 'Ekim kara' was collected from the collection in the Sun valley near the town Sudak. Young leaves were dried using silicagel at 55 °C.

Morphological analysis: 52 cultivars were characterized using 48 OIV descriptors (Maul *et al*. 2012). 23 cultivars were phenotyped using the BBCH scale. For these 23 accessions eno-carpological and eno-chemical traits were measured according to the protocols proposed during the COST Action FA1003 (Rustioni *et al*. 2014)

DNA extraction: DNA extraction and microsatellite analysis were done at the laboratory "Diversity and genome of cultivated plants", Centre INRA de Montpellier (France). 100 mg of plant material were used for DNA extraction. Genomic DNA was extracted using Qiagen DNeasy plant mini kit protocol (QIAGEN, Germany) with minor modifications (Laucou *et al*., 2011).

Chloroplast microsatellite analysis (cpSSR): Chloroplast DNA variation was analysed at three cpSSR loci: ccmp3, ccmp5, ccmp10 (Arroyo-García *et al.* 2006.).

Microsatellite analysis (SSR): A set of 22 nSSR markers was used for grape diversity analysis (This *et al*. 2004, Laucou *et al*. 2011). DNA was twice diluted before use. Amplifications were performed by adding 20 ng of genomic DNA to a 20 µL PCR mixture. All forward primers were labeled with the fluorochromes 6-FAM, NED or HEX (Applied Biosystems). PCR and electrophoresis were performed as described by Laucou *et al*. (2011). Eight multiplex PCR were used. For all multiplex except one annealing temperature was 56 °C. For multiplex containing primers VMC1b11 and VMC4f3 the temperature was 60 °C (Laucou *et al*. 2011). The separation of SSR fragments was carried out in an ABI 3130 Genetic Analyser (AB) with POP 7 polymer (Applied Biosystems, CA). Three sequencing runs were used. Peak sizes were determined using Genescan v.3.7 software. The Internal size marker GENESCAN HD 400 POX was used as an internal marker. Amplified fragments were sized with the software GeneMapper (version 3.5).

Data analysis: Standard genetic parameters (allele frequencies, expected and observed heterozygoty (He and Ho), deviations from Hardy Weinberg proportions) were calculated using the POPGENE software (version 1.32). The program DARwin (version 5.0.158, http://darwin.cirad.fr/darwin/) was employed to calculate Nei's genetic distance with standard deviations (1000 bootstraps) and to construct neighbour-joining tree. SSR profiles were compared with the INRA genetic resource database which contains more than 7,000 accessions.

Results and Discussion

Morphological analysis: Vineyards have been an important part of the Crimean landscape, as well as its culture. About 70 % of the cultivars are used for wine, the remaining ones for table grape production. Only 20 autochthonous cultivars are nowadays cultivated on industrial vineyards in Crimea. The other cultivars are maintained only in the Magarach's ampelographical collection. Some of them are represented in the collection only by one individual plant. During the project, the list of Crimean old grapevine cultivars was specified based on results of ampelographic characterization and genetic identification. Unfortunately, we can conclude that more than 70 % of Crimean cultivars are endangered.

During the 2012-2013 vegetative period, 52 old grapevine cultivars were characterized using 48 OIV descriptors and BBCH scale. Ampelographic characteristics of young shoot, mature leaf, bunch and berry were investigated. Ripening time, grape yield, mechanical characteristics of bunch and berry were determined by standard methods of measurement suggested in the frame of the COST Action FA1003 project. Analyses of the ampelographic traits of the studied grape cultivars showed that the genotypes considerably differ by their main morphological, biological and enological characteristics. The study of morphological similarity revealed the existence of three groups. This grouping was also confirmed by the analysis of genetic distances on SSR data.

Enological and eno-carpological traits of 19 Crimean cultivars and 3 controls ('Saperavi', 'Rkatsiteli' and 'Cabernet Sauvignon') as well as their morphological characters have been described. Differences in anthocyanin and phenolic substances were detected. Analysis of phenolics in the berry skin of the studied cultivars revealed their highest accumulation in 'Kefessia' (3347.7 mg·L^{-1}), followed by 'Kok Pandas' (3287.4 mg·L^{-1}) and 'Manjil al' (2940.5 mg·L^{-1}). This was considerably higher than what was found in the control grapes 'Rkatsiteli' (728.6 mg·L^{-1}), 'Cabernet Sauvignon' (1310.5 mg·L^{-1}) and 'Saperavi' (1554.5 mg·L^{-1}). Large amounts of phenolics were also found both in berry skin and seeds of the former two studied cultivars. The highest anthocyanin levels were determined in the berry skin of 'Kefessia' (1035.1 mg·L^{-1}), which was more than in 'Cabernet Sauvignon' (862.8 mg·L^{-1}) and yet slightly lower than in 'Saperavi' (1146.5 mg·L^{-1}). More detail morphological, eno-carpological and eno-chemical analysis will be present in future publication.

Nuclear microsatellite analysis: A total of 76 accessions of Crimean autochthonous grape cultivars were studied using 22 SSR loci. A total of 238 alleles for 22 nSSR loci were detected (Tab. 1). The number of alleles per locus ranged from 5 (VVIb01 and VVIn16) to 19 (VMC4f3 and VVIv67) with a mean number of 10.8. The analysis revealed a level of expected heterozygosity (H_E) ranging from 0.37 (VVIn73) to 0.88 (VMC1b11, VMC4f3 and VVIp31). The average gene diversity was 0.73. The observed heterozygosity varied from 0.37 (VVIn73) to 0.90 (VMC4f3) with an average of 0.76. The most frequent alleles in this study were VVIb01-294 (59 %), VVIh54-165 and VVIn16-149 (58 %), VVIn73-263 (78 %), VVMD24-206 (69 %), VVMD28-257 (51 %).

Table 1

Statistical results for 22 microsatellite markers used in the present study, namely: observed number of alleles (na), effective number of alleles (ne), Shannon's Information index (I), observed heterozygosity (Ho), expected heterozygosity (He), and average heterozygosity (Ave Het)

Locus	na	ne	I	Ho	He	Ave Het
VMC1b11	11	7.8	2.16	0.88	0.88	0.87
VMC4f3	19	7.7	2.34	0.90	0.88	0.87
VVIb01	5	2.2	0.95	0.63	0.54	0.54
VVIh54	15	3.1	1.73	0.73	0.68	0.67
VVIn16	5	2.2	1.00	0.55	0.56	0.55
VVIn73	7	1.6	0.83	0.37	0.37	0.37
VVIp31	13	8.1	2.27	0.87	0.88	0.88
VVIp60	10	3.8	1.60	0.79	0.75	0.74
VVIq52	6	3.5	1.33	0.67	0.72	0.71
VVIv37	12	5.8	1.98	0.88	0.83	0.83
VVIv67	19	5.7	2.21	0.79	0.83	0.83
VVMD21	8	4.1	1.61	0.86	0.76	0.76
VVMD24	10	2.0	1.12	0.46	0.49	0.49
VVMD25	11	4.6	1.70	0.89	0.79	0.78
VVMD27	8	6.2	1.90	0.87	0.85	0.84
VVMD28	13	3.0	1.56	0.69	0.67	0.67
VVMD32	9	5.4	1.82	0.87	0.82	0.82
VVMD5	10	6.4	1.98	0.87	0.85	0.84
VVMD7	9	3.1	1.41	0.70	0.68	0.68
VVS2	12	4.3	1.81	0.72	0.77	0.77
VrZAG62	12	7.1	2.15	0.87	0.86	0.85
VrZAG79	14	4.4	1.95	0.79	0.78	0.77
Mean	10.8	4.7	1.70	0.76	0.74	0.73
St. Dev	3.8	2.0	0.45	0.15	0.14	0.14

SSR analysis confirmed the differentiations and similarities among the Crimean cultivars. In our study the dendrogram constructed on the basis of 22 SSR loci analysis shows two groups of synonyms, where local cultivars known with different names display identical SSR profiles (Fig. 2). The comparison of SSR-profiles and morphological data of 'Manjil al', 'Shabash' and 'Shabash Krupnoyagoddnyi' revealed the same SSR profile and similar morphological characteristics (cluster 81). Identical profiles were also detected for cultivars 'Ekim kara' and 'Krona' (cluster 79). A high genetic relation has been detected between 'Khachador', 'Biyas aibatly' and 'Polkovnik izyum' (cluster 78). Moreover, these cultivars showed a high similarity with 'Colonel (Isioum)'. Several synonyms were identified based on the comparison of the microsatellite profiles' data with those found in INRA database: 'Kutlakskii chernyi' = 'Catalan', 'Firski rannii'='Firsky précoce', 'Dardagan' = 'Hagnos Zöld', 'Kapitan Yani kara' = 'Rindjaga', 'Kornishon crymskii' = 'Ciliegiulo nero', 'Khalil izyum' = 'Kara oglan faux', 'Zerva' = 'Seibel 13666', 'Abla Aganyn izyum' = 'Nuyu isioum'. Besides, the «Kokur» group varieties shared identical profiles, with only one difference. Based on ampelographical data, the cultivars 'Kokur polurassechennyi' and 'Kokur rassechennyi' are var-

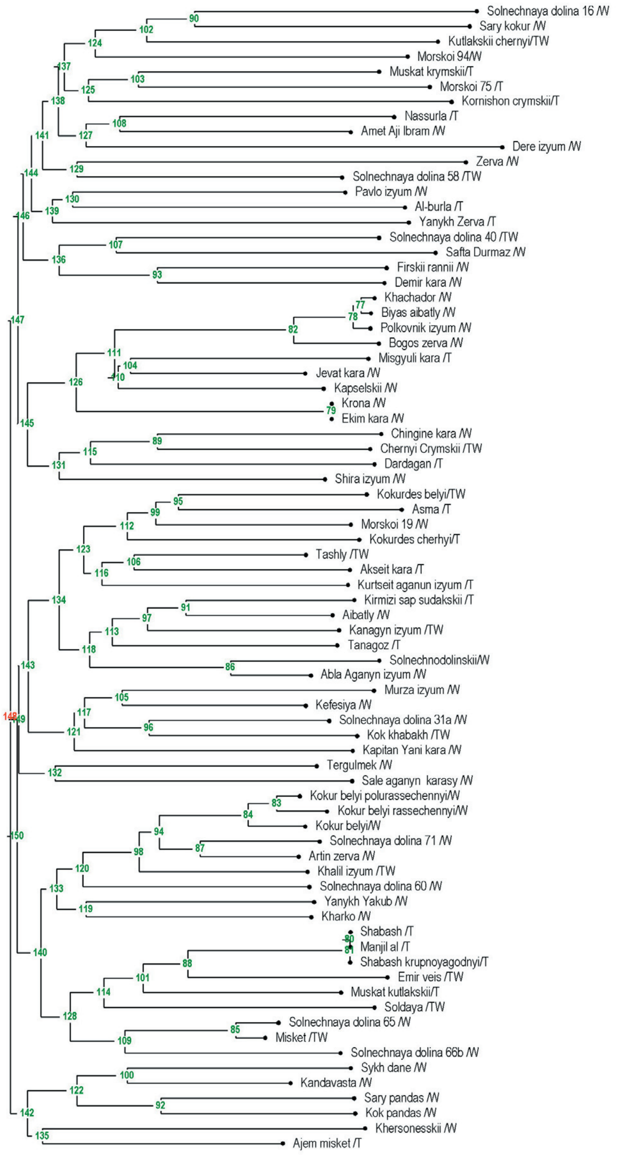

Fig. 2: Dendrogram of 76 Crimean grapevine cultivars genotyped at 22 nSSR loci. The dendrogram was build using the Weighted Neighbour-Joining method with the Darwin software. Number of cluster is designated by cipher. The indication of the use of the fruit is preceded by a slash mark: wine - W, table - T, TW - table wine.

iants of 'Kokur belyi' (Gramotenko, 1970). As a result, the 76 investigated accessions of the Crimean autochthonous cultivars were found to belong to 57 unique profiles.

Analysis of genetic diversification revealed a tendency for the differentiation based on the use of the fruit: wine (W), table (T), table-wine (TW). The cultivars were grouped into clusters having subclusters with i) W and TW); ii) T and TW (Fig. 2).

Results of ampelographic characterization of Crimean local cultivars showed the high level of variability. Comparison of Crimean cultivars SSR profiles with the Eu-Vitis and the French database INRA confirmed that majority of them are unique and exist only in Crimea. The results were uploaded into the European Vitis Database (www.eu-vitis.de).

Chloroplast microsatellite analysis: The analysis of haplotype diversity shows that all chloroplast loci are polymorphic within the 76 cultivars. A total of 8 alleles were detected. Three cpSSR loci combine in a total of 5 different haplotypes (Tab. 2). Of them haplotypes A, B, C, D were identified as indicated in Arroyo-Garcia *et al*. (2006). These haplotypes corresponded to haploptypes IV, II, V, I that were identified using two cpSSR loci (ccmp3, ccmp10) by Imazio *et al.* (2006). Only one haplotype, identified for 'Solnechnaya dolina 31a' was unique. Haplotype II (B) was the most represented (29 out of 76). It is interesting to note that this haplotype, as Imazio *et al.* (2006) note, is mostly represented in Italian accessions. It confirms the supposition that they were imported by the Genoese and the Turks and came from West European origin (Negrul 1946).

Table 2

Haplotypes identified in the accessions of the Crimean cultivars using chloroplast SSR loci ccmp3, ccmp5 and ccmp10. The first two coloumns display the number and percent of the haplotypes belonging to the different haplogroups, among the 76 studied accessions. These haplotypes corresponded to haploptypes A, B, C, D as identified by Arroyo-Garcia *et al.* (2006) and haploptypes IV, II, V, I as identified using two cpSSR loci (ccmp3, ccmp10) by Imazio *et al.* (2006). One identified haplotype was unique

Number of accessions	%	Classification of haplotypes	
		Arroyo-Garcia *et al.* 2006	Imazio *et al.* 2006
1	1.3	not present	not present
13	17.1	A	IV
29	38.2	B	II
10	13.2	C	V
23	30.1	D	1

Haplotype I, which is highly represented in Caucasian accessions, as indicated Imazio *et al.* (2006), was identified in 30 % of the Crimean cultivars. Some authors consider that many Crimean varieties were imported from Caucasia (Negrul 1946, Ivanov 1947). Furthermore, a route of the Silk Road passed through the Crimea. At the end of the 1st century BC, the Silk Road divided where the River Don empties into the Sea of Azov. Routes originating from that place led to all large cities of the states located on the shores of the Sea of Azov and the Black Sea, such as Phanagoreia, Cafa (modern Feodosia), Olvia, Sugdeia (Sudak) and Chersonesus (Fig. 1).

Haplotype IV was identified in 10 cultivars. This haplotype was the rarest and completely absent in the group of East European varieties (Imazio *et al*. 2006).

Conclusions

Origin of Crimean grape cultivars is a very complex process. Comparing morphobiological traits of some autochthonous varieties of the Crimea and of the revealed variants of *Vitis vinifera* ssp. allows us to suggest that a number of local cultivars originate in the Crimea. For the major part, they are wine cultivars: 'Khersonesskii', 'Manjil al', 'Kastel chernyi', 'Ekim kara', 'Jevat kara' etc. (Malikov 1968). Thus, judging by morphological traits, the variety 'Khersonesskii' reminds of wild-growing grape and is found in the region near an ancient Greek city Chersonesus (now Sevastopol) (Dashkevich 1966). Onward, concurrent with the development of ancient Greek colonies on the seacoast of Crimea in the 7th-6th centuries BC, new grape varieties were imported to the area. For instance, the cultivars 'Sary pandas', 'Sary kokur', 'Kokur belyi', 'Kandavasta' have Greek names (Ivanov 1947, Korzhinskii 1904, Negrul 1954). It is possible that the formation of grape cultivars of the Crimea has been affected by a number of factors, such as natural selection, artificial selection and hybridization of local and imported varieties. These results suggest that historical movement of grapevine was important in shaping the structure of the grapevine genepool. This study will make available additional information about the genetic diversity and the relationship among Crimean cultivars. The genetically rich and diversified genepool of Crimean cultivars represents valuable material sources for future sustainable breeding and improvement of grapevine.

Acknowledgements

The authors wish to thank P. This, F. Lefort, J. M. Martinez-Zapater, R. Arroyo-Garcia, O. Failla and E. Maul for valuable advice and assistance in doing research. This research was supported by EU program COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" and by the Russian Foundation for Basic Research (grant №15-29-02715).

References

Arroyo-García, R.; Ruiz-García, L.; Bolling, L.; Ocete, R., Lopez, Arnold, C.; Ergul, A.; Soylemezoglu, G.; Uzun, H. I.; Cabello, F.; Ibáñez, J.; Aradhya, M. K.; Atanassov, A.; Atanassov, I.; Balint, S.; Cenis, J. L.; Costantini, L.; Gorislavets, S.; Grando, M. S.; Klein, B. Y.; McGovern, P. E.; Merdinoglu, D.; Pejic, I.; Pelsy, F.; Primikirios, N.; Risovannaya, V.; Roubelakis-Angelakis, K. A.; Snoussi, H.; Sotiri, P.; Tamhankar, S.; This, P.; Troshin, L.; Malpica, J. M.; Lefort, F.; Martinez-Zapater, J. M.; 2006: Multiple origins of cultivated grapevine (*Vitis vinifera* L ssp *sativa*) based on chloroplats DNA polymorphims. Mol. Ecol. **15**, 3707-3714.

Carter, J.; Pashkevich, G.; 2004: Cultivated plants of the Northern Pontos during the Greek colonization. In: Development of national programmes on plant genetic resoursers in Southeastern Europe. Sec. Proj. Met. 48-49. Yalta, Ukraine, IV&W "Magarach".

Dashkevich, A. B.; 1966: Cultivar 'Khersonesskii'. In: Ampelography of USSR. A little widespread cultivars of grapes. Food-Proc. Ind. **3**, 338-339.

Gismondi, A.; Impei, S.; Di Marco, G.; Crespan. M.; Leonardi, D.; Canini, A.; 2014: Detection of new genetic profiles and allelic variants in improperly classified grapevine accessions. Genome **57**, 111-118.

Gramotenko, P. M.; Matvienko, N. N.; Pestretsov, V. V.; 1979: The Perspective aboriginal grape cultivars of Crimea. Winemaking Winegrowing USSR **1**, 36-37.

Gramotenko, P. M.; 1970: Kokyr belyi polurassechennyi. Ampelography of USSR. Help Volume. Food-Proc. Ind. **3**, 338-339.

Heuertz, M.; Goryslavets, S.; Hausman, J. F.; Risovanna, V.; 2008: Characterization of grapevine accessions from Ukraine using microsatellite markers. Am. J. Enol. Vitic. **59**, 169-178.

Imazio, S.; Labra, M.; Grassi, F.; Scienza, A.; Failla, O.; 2006: Chloroplast microsatellites to investigate the origin of grapevine. Genet. Res. Crop Evol. **53**, 1003-1011.

Ivanov, A. A.; 1947: The Crimean Native Varieties of Grapes, 79. Crimea-Print, Simferopol.

Khrapunov, I. N.; Gertsen, A. G.; 2010: From Cimerian to Crimchak. The people of Crimea since the most ancient times till the end of XVIII centuries, 286. Dolya, Simferopol.

Korzhinskii, S. I.; 1904: Ampelography of the Crimea. Main Management of the Appanage.

Labra, M.; Moriondo, G.; Schneider, A.; Grassi, F.; Failla, O.; Scienza, A.; Sala, F.; 2002: Biodiversity of grapevines (*Vitis vinifera* L) grown in the Aosta Valley. Vitis **41**, 89-92.

Laucou, V.; Lacombe, T.; Dechesne, F.; Siret, R.; Bruno, J. P.; Dessup, M.; Dessup, T.; Ortigosa, P.; Parra, P.; Roux, C.; Santoni, S.; Vares, D.; Peros, J. P.; Boursiquot, J. M.; This, P.; 2011: High throughput analysis of grape genetic diversity as a tool for germplasm collection management. Theor. Appl. Genet. **122**, 1233-1245

Lefort, F.; Massa, M.; Goryslavets, S.; Risovanna, V.; Troshin, L.; 2003: Genetic profiling of Moldavian, Crimean and Russian cultivars of *Vitis vinifera* L. with nuclear microsatellite markers. In: A. Lonvaud-Funel, G. de Revel, G. Darriet (Eds): Oenologie, 71-73. 7éme Symp. Int. d'Oenol. Ed. Tec & Doc, Paris.

Malikov, B. M.; 1968: Wild-growing grapes on ancient and medieval settlements of Crimea as an initial material for selection and replenishment of high-quality fund: The author's abstract, 21. Kishinev.

Maul, E.; Sudharma, K. N.; Kecke, S.; Marx, G.; Müller, C.; Audeguin, L.; Boselli, M.; Boursiquot, J. M.; Bucchetti, B.; Cabello, F.; Carraro, R.; Crespan, M.; de Andrés, M. T.; Eiras Dias, J.; Ekhvaia, J.; Gaforio, L.; Gardiman, M.; Grando, S.; Agyropoulos, D.; Jandurova, O.; Kiss, E.; Kontic, J.; Kozma, P.; Lacombe, T.; Laucou, V.; Legrand, D.; Maghradze, D; Marinoni, D.; Maletic, E.; Moreira, F.; Muñoz-Organero, G.; Nakhutsrishvili, G.; Pejic, I.; Peterlunger, E.; Pitsoli, D.; Pospisilova, D.; Preiner, D.; Raimondi, S.; Regner, F.; Savin, G.; Savvides, S.; Schneider, A.; Sereno, C.; Simon, S.; Staraz, M.; Zulini, L.; Bacilieri, R.; This, P.; 2012: The European *Vitis* Database (www.eu-vitis.de) – a technical innovation through an on-line uploading and interactive modification system. Vitis **51**, 79-86.

Negrul, A. M.; 1960: Archeological finds of seeds of grapes. Soviet Archeol. **1**, 111-119.

Negrul, A. M.; 1958: Wild-growing grapes of Crimea and its connection with local varieties, 39-50. News TCXA, Moscow.

Negrul, A. M.; 1946: Origin of cultural grapes and its classification. In: A. M. Frolov-Bagreev (Ed.): Ampelography of the USSR, 159-216. Pischepromizdat, Moscow.

Rustioni, L.; Maghradze, D.; Popescu. C. F.; Cola, G.; Abashidze, E.; Aroutiounian, R.; Brazao, J.; Coletti, S.; Cornea, V.; Dejeu, L.; Dinu, D.; Eiras Dias, J. E.; Fiori, S.; Goryslavets, S.; Ibanet, J.; Kocsis, L.; Lorenzini, F.; Maletic, E.; Mamasakhlisashvili, L.; Margaryan, K.; Mdinaradze, I.; Memetova, E.; Montemayor, M. I.; Munoz-Organero, G.; Nemeth, G.; Nikolaou, N.; Pastore, G.; Preiner, D.; Raimondi, S.; Risovanna, V.; Sakaveli, F.; Savin, G.; Savvides, S.; Schneider, A.; Schwander, F.; Spring, J. L.; Ujmajuridze, L.; Zioziou, E.; Maul, E.; Bacilieri, R.; Failla, O.; 2014: First results of the European grapevine collections' collaborative network: validation of a standard eno-carpological phenotyping method. Vitis **53**, 219–226

Sefc, K. M.; Lopes, M. S.; Lefort, F.; Botta, R.; Roubelakis-Angelakis, K. A.; Ibáñez, J.; Pejíc, I.; Wagner, H. W.; Glössl, J.; Steinkellner, H.; 2000: Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor. Appl. Genet. **100**, 498-505.

This, P.; Jung, A.; Boccacci, P.; Borrego, J.; Botta R.; Costantini, L.; Crespan, M.; Dangl, G. S.; Eisenheld, C.; Ferreira-Monteiro, F.; Grando, M. S.; Ibáñez, J.; Lacombe, T.; Laucou, V.; Magalhães, R.; Meredith, C. P.; Milani, N.; Peterlunger, E.; Regner, F.; Zulini, L.; Maul, E.; 2004: Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor. Appl. Genet. **109**, 1448-1458.