Genetic Profiling Of Moldavian, Crimean and Russian Cultivars Of *Vitis Vinifera* L. with Nuclear Microsatellite Markers

FRANÇOIS LEFORT[1], SVETLANA GORISLAVETS[2], VALENTINA RISOVANNAYA[2] & LEONID TROSHIN[3]

[1]Laboratory of Biotechnology and Applied Genetics, Ecole d'Ingénieurs de Lullier, Haute Ecole Spécialisée de Suisse Occidentale, 150, route de Presinge, 1254 Jussy, Switzerland.
[2]Institute of Vine and Wine Magarach, 31 Kirov St. 98600 Yalta, Crimea, Ukraine.
[3]Viticulture Department, Kuban State University of Agriculture, 350044 Krasnodar, Kuban, Russia

Corresponding author : Lefort, F., E-mail : francois.lefort@etat.ge.ch

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Introduction

Microsatellite markers have been proved to be a useful tool for assessing genetic identities and genetic relationships between grapevine gene pools (for a review, see Sefc et al., 2001). Characterization of Moldavian, Russian and Crimean genetic resources of grapevine sources with the use of nuclear microsatellite nuclear markers is reported here for the first time.

The 52 Crimean and 27 Moldavian cultivars, included in this study, are conserved in the ampelographic collection of the Institute of Wine and Vines Magarach in Yalta, Crimea, Ukraine and represent a major part of the grapevine genetic resources from these provenances. Crimean cultivars are cultivars from the region of Crimea and not from other regions of Ukraine. The 24 Russian cultivars, are conserved in the new ampelographic collection of Russia, located at the University of Agriculture of the Kuban state in Krasnodar. Cultivars were selected as being potentially the most ancient cultivars cultivated in these regions, without prejudice of their native or foreign origin, since it could be likely for historical reasons that some Greek, Turkish or Caucasian cultivars would have been transmitted to these regions.

Genetic profiling of these cultivars was carried out with 9 nuclear microsatellite loci previously characterized: VVS2, ssrVrZAG21, ssrVrZAG47, ssrVrZAG62, ssrVrZAG 64, ssrVrZAG79, ssrVrZAG83, ssrVvUCH11 and ssrVvUCH29.

These loci have already been used in similar works of genetic characterization in other European countries. Consequently, allele sizing carried out in our laboratory was standardized with profiling results obtained at the same loci by Sefc at the University of Agriculture of Vienna, Austria and by Lefort at the University of Heraklion in Greece. Standardization allowed to compare these Crimean, Moldavian and Russian grapevine cultivars with Western European and Greek genetic resources already characterized at the same loci.

Materials and Methods

Plant material:

Leaves of *Vitis vinifera* L. cultivars were collected from the ampelographic collections of the Institute of Wine and Vines, Magarach, Yalta, Crimea, Ukraine and of the Viticulture Department of the University of Agriculture of the Kuban state, Krasnodar, Russia.

DNA extraction:

DNA was extracted from 100 to 150 mg fresh weight of leaf tissue according to a previously described micro-method of DNA purification developed for hardwood species and modified for *Vitis* species. (Lefort and Roubelakis-Angelakis, 2001)

Microsatellite PCR and microsatellite profile analysis:

Amplification primer sequences for 9 nuclear microsatellite loci from *Vitis riparia* (Sefc et al., 1999), ssrVrZAG 21, ssrVrZAG 47, ssrVrZAG 62, ssrVrZAG 64, ssrVrZAG 79, ssrVrZAG 83, and from *Vitis vinifera*, VVS2 (Thomas et al., 1994), UCH11, and UCH29 (Lefort et al., 2002), were used for DNA amplification.

PCR amplifications were carried out in 96-well propylene plates in 20 µl final volume reaction mixtures in a Gradient Mastercycler (Eppendorf, Germany). PCR reactions were as follows: 1 µM of each primer, 100 µM of each dNTPs (Biofinex, Praroman, Switzerland), 1.5 mM $MgCl_2$ in the buffer 75 mm Tris-HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2 SO_4$, 0.5 units Taq polymerase (Biotools, Madrid, Spain) and 50 ng DNA template. The forward primer in each case was labeled with the Cy5 fluorochrome (Amersham Biosciences, UK). The following thermal cycling protocol was applied for all loci: 95°C for 5 min, 10 cycles of 15 s at 50°C, 15 s at 94°C, followed by 23 cycles of 15 s at 50°C, 15 s at 89°C and terminated immediately at 4°C, except for ssrVrZAG64, of which the annealing temperature was 58°C. PCR product analysis was carried out on Reprogel template (Amersham Biosciences) in an ALFExpress 2 DNA Sequencer (Amersham Biosciences), and alleles were sized with the software Allele Locator (Amersham Biosciences). PCR samples were run along with internal size markers 100 bp and 300 bp. Additionnally, external markers scale (50 bp –350 bp) and home made gold markers for each locus were run in the most peripheral wells. Sizing was standardized for all loci with previous works (Sefc et al., 2000, 2002; Lefort and Roubelakis, 2000; Lefort et al., 2001) using the same markers set, in order to allow an easy comparison with other *Vitis vinifera* germplasm. The phenogram presented in figure 1 was obtained by using MICROSAT software (Minch et al., 1997) for calculating genetic distance in [-log(proportion of shared alleles)]. The distance matrix obtained from MICROSAT was processed with KITSCH from the PHYLIP package (Feselstein, 1989) and the phenogram was drawn with TREEVIEW (Page, 1996). Observed and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$) (Nei, 1973), probability of identity ($PI = \Sigma p_i^4 + \Sigma\Sigma [2p_i p_j]^2$) (Paetkau et al., 1995), and probability of null alleles ($r = [H_e - H_o] / [1 + H_e]$) (Brookfield, 1996) were calculated with IDENTITY 1.0 (Wagner and Sefc).

Results and Discussion

Cultivars included in the present study are shown in table 1. According to the transliteration in Latin alphabet, it appears that several cultivars seem to have a name of Turkish origin, which is not a proof of Turkish origin but could indicate that these cultivars could have been transmitted from Turkey or former Turkish dependences. Analysis of microsatellite profiling are given in table 2. The total number of alleles found in these 103 cultivars was high at 105 alleles and consequently the mean number of alleles per locus was high at 11,66 which was much higher than those previously recorded in other gene pools with the same set of markers (Lefort and Roubelakis-Angelakis, 2001; Sefc et al. 2000). Heterozygosity was high and ranged between 0.71 and 0.93, though the estimated frequency of null alleles was surprisingly close to 0.05 at 2 loci VVS2 and ssrVrZAG79, which resulted from a slight excess of homozygous cultivars at these loci. The average heterozygosity was high at 0.80 and expressed an overall high genetic diversity, which was also expressed by a low average genetic similarity of about 37% as calculated from the distance matrix. As shown on figure 1, microsatellite profiling at 9 loci was powerful enough to discriminate 103 cultivars in 102 single identity profiles. Most of the cultivars clustered in groups of branches according to their geographic origins. Russian cultivars seem to be of mixed origins, with some groups closer to Moldavian cultivars while others were closer to Crimean cultivars. Only one pair of synonyms were found among these 103 cultivars and they were two Crimean cultivars. Biyas aibatly and Khachador. Possible parent relationships were only found for 5 combinations of cultivars with four of them involving Moldavian cultivars, which would need further investigation at more loci. Such a low level of possible parent relationship at only 9 loci is congruent with the high observed diversity and could suggest that a large part of these resources were from diverse origins. No synonyms were found between cultivars from these 3 provenances and 305 other cultivars from France, Greece, Switzerland and Albania (data not shown) when comparison were made at 8 loci out of nine.

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Table 1: Moldavian, Crimean and Russian cultivars of *Vitis vinifera* L. used in the present study. Names of Crimean and Russian cultivars were transliterated from Ukrainian and Russian Cyrillic alphabets according to the appropriate schemes of transliteration.

RUSSIE	CRIMÉE
Agadai	Abla aganyn izium 173
Ag-izyum	Adgem misket 174
Alyi terskii	Aibatly 175
Asyl Kara	Akseit kara 176
Bulanyi	Alburla 177
Buryi	Amet Adgi Ibram 178
Cikrah	Artin zerva 179
Cimlijanszki chernyi	Asma
Gyulyabi Dagestanski	Biyas aibatly
Kaitangi	Bogos zerva
Klinchatyi	Cherny kuymski
Krasnostop zolotovski	Cornichon crymski
Kumshatskii belyi	Crona
Lesnoi belyi maraginski	Dardagan
Makhbor Tsibil	Demir kara
Makhrovatchic	Dere izium
Narma	Dgevat kara
Plechistik	Firski ranni
Pukhlyakovski	Kapitan Yani kara
Rish Baba	Kapselski
Shavrony	Kastel chernyi
Sibirkovyi	Kefesia
Tygys	Khachador
Varyoshkin	Khalil izium
	Khersonesski
MOLDAVIE	Kirmisi sap sudakski
Adgi	Kok khabakh
Akkermanski chernyi	Kok pandas
Ali-ali negru	Kokur belyi
Alimshak	Kokurdes belyi
Alvarna	Kokurdes chernyi
Briazy	Kovalevka
Cabasma	Kurtseit aganyn izium
Cabassia	Mangil al
Chorcutsa rosove	Misgiuli kara
Copchak	Misket
Fet frumos	Murza izium
Fetiaska niagre	Nasurla
Galabura	Pavlo izium
Galbena	Safta durmaz
Gordin verde	Sale aganyn kara
Moldavski belyi	Sary kokur
Muscat bessarabski	Sary pandas
Muscat de Codru	Shira izium
Muscat moldavski	Soldaya
Seyna	Solnechnodolinski
Sgigarda	Sykh dane
Sgigardai krasnoplodnyi	Tanagoz
Tidveska	Tashly
Tiras	Tergulmek
Tsisa caprian	Yanykh zerva
Turba plotnyi belyi	Zerva
Turba rykhlyi	

Table 2: Analysis of 102 profiles found in 103 cultivars: Number of alleles, observed and expected heterozygosity, probability of identity, and estimated frequency of null alleles at 9 nuclear microsatellite loci.

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Loci	Number of alleles	H_o	H_e	Probability of identity (PI)	Estimated frequency of null alleles
VVS2	11	0.7254	0.8259	0.0952	0.0550
SsrVrZAG21	14	0.9313	0.8745	0.0533	- 0.0303
SsrVrZAG47	10	0.8627	0.815	0.0751	- 0.0063
SsrVrZAG62	10	0.9117	0.8352	0.0883	- 0.0417
SsrVrZAG64	9	0.8431	0.8156	0.1128	- 0.0151
SsrVrZAG79	12	0.7843	0.7862	0.105	0.0011
SsrVrZAG83	9	0.6274	0.6610	0.3029	0.0202
UCH11	11	0.7156	0.8292	0.0932	0.0620
UCH29	19	0.8627	0.8187	0.0827	- 0.0241
	105 alleles		0.8068		
Mean	MNA = 11.(	0.8071	0.8068	PI for all loci 9.35×10^{-10}	

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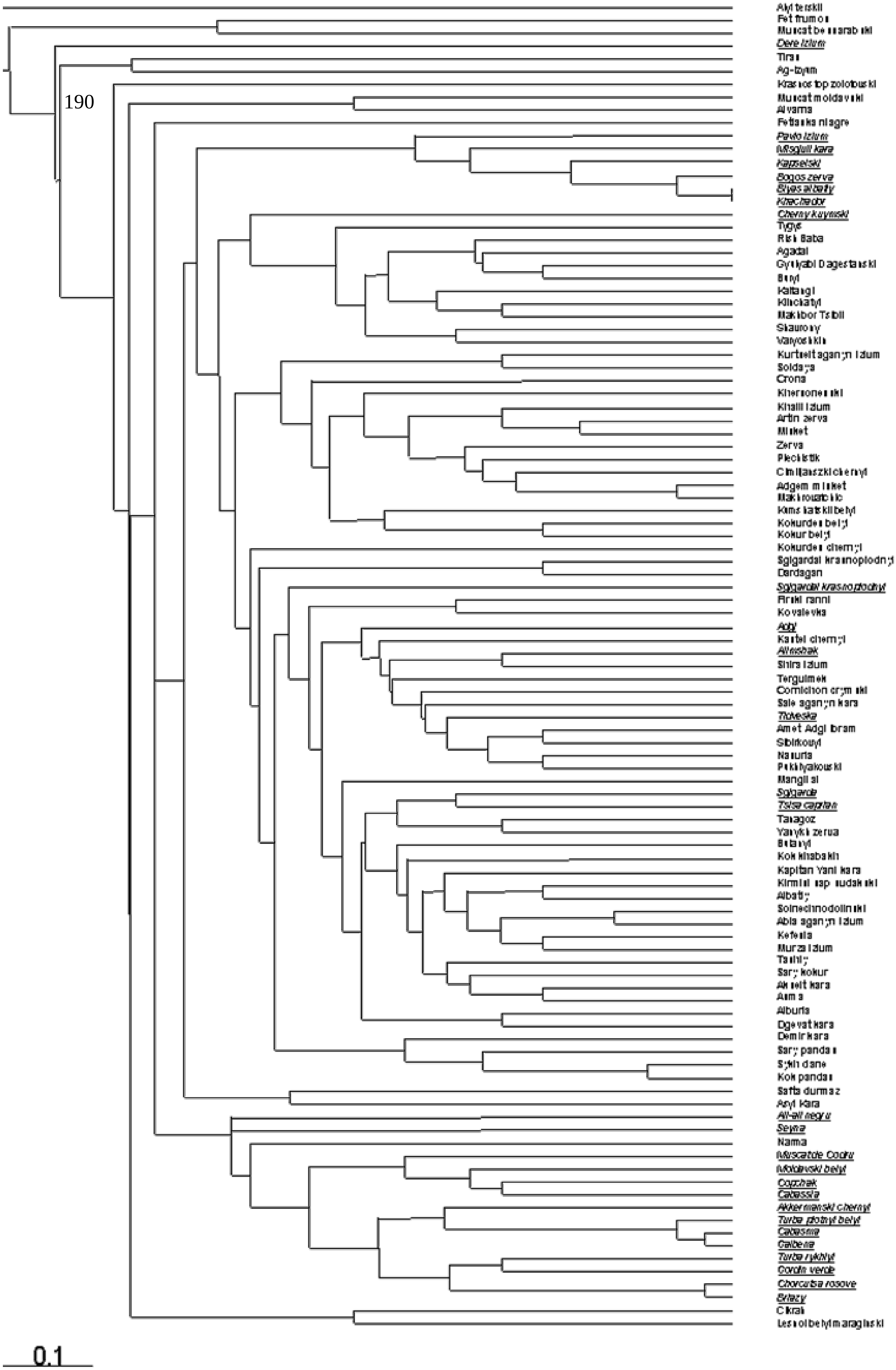
Figure 1. Phenogram of Moldavian, Ukrainian and Russian grapevine cultivars. Moldavian cultivars are shown in italics underlined, Crimean cultivars are shown in bold and Russian cultivars are shown in black. The genetic distance used was (-log [proportion of shared alleles]) and the scale is a function of this distance.



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Alyi terskii
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Krasnostop zolotovskii
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Alvarna
Fetiaska niagre
Pavlo izium
Misgurli kara
Kapselski
Bogos zerva
Ryas albatly
Kirachadur
Cherny krymski
Tygys
Rish Baba
Agadai
Gyulyabi Dagestanski
Beryi
Kaltangi
Kilichatyi
Makibor Tsibil
Skaurony
Kurtseit agasyn izium
Soldaya
Crona
Khersonesskii
Khalil izium
Artin zerva
Misket
Zerva
Pleckistik
Cimljanskii chernyi
Adgem misket
Makhrouatokle
Kimsaiatskii belyi
Kokurdes belyi
Kokur belyi
Kokurdes chernyi
Sgigardai krasnoplodnyi
Dardagan
Sgigardai krasnoplodnyi
Firiki ranni
Kovalevka
Adgi
Kastel chernyi
Almshak
Shira izium
Tergulmek
Cornichon crymskii
Sale agasyn kara
Troveska
Amet Adgi Ibram
Sibirkovyi
Naburis
Pekiiyakovskii
Mangilsi
Sgigarda
Tsitsa capritei
Taragoz
Yanyki zerva
Betanyi
Kokkisabakin
Kapitan Yani kara
Kirmizi sap sudakskii
Albatiy
Solnechnodolinskii
Abla agasyn izium
Kefesia
Murza izium
Tashliy
Sary kokur
Akseit kara
Asma
Alburla
Dgevat kara
Demir kara
Sary pandas
Sykh dane
Kok pandas
Safta durmaz
Aryi kara
Ali-ali negru
Seyna
Narma
Muscat de Codru
Moldavskii belyi
Copchak
Cabassia
Akkermanski chernyi
Turba plotnyi belyi
Cabasma
Galbena
Turba rykhlyi
Coarna verde
Chorcutsa rosove
Brlazy
Cikrak
Lesnoi belyi maraguskii
0.1