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Identification and characterization of Romanian grapevine genetic resources

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Summary

The research focused on old Romanian varieties of local distribution, with very limited growing area and also on those nowadays neglected, to identify, characterize and compare these cultivars with one another and on a larger geographic scale. Ampelografic characterization with 48 descriptors, photographs of shoot tip, leaf and bunch and genotyping using 13 SSR markers were applied together with literature references to confirm trueness to type. The sixty one accessions belonged to fifty one varieties. New synonymies were detected (e.g. 'Negru românesc' = 'Bătută neagră'; 'Cârcioasă' = 'Balint weiss'; 'Galbenă măruntă' = 'Kakotrygis'), assignments were modified (e.g. 'Galbenă uriaşă' = 'Mirkovaca' and ≠ 'Galbenă de Odobeşti' as previously assumed), misnomers identified and unique genotypes were detected which had never been described before. Molecular data supported previous pedigree relationships and allowed new parent-offspring hypotheses to be formulated. The results were shown to be useful for updating the information on old Romanian grapevine germplasm.

K e y w o r d s : SSR; genotyping; autochthonous cultivars; synonyms; germplasm.

Introduction

There is a long history of grapevine cultivation and wine production on Romanian territories, documented from the 7th century BC onwards (Constantinescu *et al.* 1970). It is today one of the most important fruit crops with a vineyard area of about 192,000 ha, 95 % for wine and 5 % for table grapes (Romanian National Office of Vine and Wine Products - NOVWP, 2016, http://www.onvpv.ro/). The first research papers with scientific descriptions of grapevine varieties were published in Romania during the 19th century (Ionescu 1868, Nicoleanu 1900) and the first ampelographic collections were established at Pietroasa (1895), Bucharest (1925), Valea Călugăreasca (1925), Huşi (1925), Drăgăşani and Miniş (1939). Other small collections were established in six research stations and four university centres aiming to maintain the highest number of grapevine varieties as possible under secure conditions. Today these collections hold around 1,381 wine and table grape accessions (17.8 % considered as Romanian varieties and clones), 42 rootstock varieties and 85 interspecific varieties for fruit production. Over time, these collections have been the source of reference plant material for thorough comparative studies and also for most research activities involving ampelographic characterization, phenology and evaluation of yield and oenological potential of grapevine varieties and clones. Proof of these intensive studies are the seven volumes of "Ampelografia Republicii Populare Romîne" published between 1959 and 1970 (Constantinescu *et al.* 1959, 1960, 1961, 1962, 1965, 1966, 1970), which are still an international reference in the field of ampelography. Description and evaluation of varieties considered of economic importance for wine production were of primary interest (Indreas and Visan 2001, Rotaru 2009). Less effort was made for old Romanian varieties of local distribution and for those nowadays neglected or with very limited growing areas.

In the last years, the ampelographic descriptions have been enriched with molecular characterisations, especially for cultivars of scientific and economic value, aiming to accurately identify the autochthonous varieties (Gheorghe *et al*. 2008, Bodea *et al*. 2009, Butiuc-Keul *et al*. 2010, Coste *et al*. 2010), or to facilitate the registration of Romanian cultivars in the European *Vitis* Database (Gheţea *et al*. 2010 and 2012).

The aim of this study was to obtain a molecular and ampelographic characterization of 61 accessions, most of them presumed autochthonous Romanian varieties, selected on the basis of the following criteria: a) varieties grown since ancient times according to old documents and turned into international cultivars due to their biological competence; b) major local cultivars, of local importance, extensively grown in Romania; c) minor cultivars, of local importance, grown especially in private vineyards; d) neglected local cultivars, at risk of extinction and retained in germplasm collections. They encompassed mainly varieties recommended for wine

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production and some table and wine/table cultivars. The research was developed within the framework of COST Action FA1003 "East-West collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding" (Failla 2015), with the aim of identifying, characterizing and comparing the selected Romanian varieties with one another and on a larger geographic scale. Knowledge of grapevine varieties provenance is useful to better understand their movement through different countries over time, even when the memory of those transfers had been lost, together with the original name of the variety. As a consequence, the information available on specific varieties may become broader and can be retained or rejected, when aware of the true identity of the cultivars under study.

Genotyping was applied to identify possible synonyms, homonyms, misnomers and unique genotypes, using a set of 13 SSR markers encompassing the nine SSR markers recommended for common use by the European project GrapeGen06 (Maul *et al*. 2012). The SSR profiles also allowed comparison of the Romanian grapevine germplasm with literature data and molecular databases, searching for synonyms with respect to neighbouring countries. Preliminary indications obtained from molecular data were then compared with available morphological information.

Material and Methods

Plant material: Sixty-one accessions (Tab. 1) were characterized, belonging to three germplasm collections: Faculty of Horticulture - University of Agronomical Science and Veterinary Medicine, Bucharest (UASVM); Research and Development Station for Viticulture and Oenology Drăgăşani-Vâlcea (RDSVO); National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti-Argeş (NRDIBH).

Ampelographic description: The ampelographic description was carried out for two or three consecutive years, in accordance with the 2nd edition of the "OIV Descriptor list for grapevine varieties and *Vitis* species" (OIV 2009) with 48 descriptors and following the standardized methodology reported in Rustioni *et al*. (2014). The morphological characteristics recorded in the three germplasm collections referred to the following aspects: 7 for young shoot (OIV 001, 003, 004, 006, 007, 008 and 016), 17 for young and mature leaf (OIV 051, 053, 067, 068, 070, 072, 074, 075, 076, 079, 080, 081-1, 081-2, 083-2, 084, 087 and 094), 15 for type of flower, bunch and berry aspects (OIV 151, 155, 202, 204, 206, 208, 209, 220, 221, 223, 225, 231, 235, 236 and 241) and 9 for phenology, growth, quality and quantity of grape yield (OIV 301, 303, 351, 502, 503, 504, 505, 506 and 508).

Genotyping and identification: Genomic DNA was isolated from 100 mg of young leaf using Qiagen DNeasy Plant mini-kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. DNA concentration and quality were checked by spectrophotometric analysis and electrophoresis in 1 % agarose gel. Thirteen SSR markers were used for genotyping: the nine proposed as common grape markers for international use within the framework of the Grapegen06 European project (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79) (Maul *et al*. 2012), plus ISV2 (VMC6e1), ISV3 (VMC6f1), ISV4 (VMC6g1) and VMCNG4b9 (Migliaro *et al*. 2013). The SSR analyses were performed following the protocol detailed in Migliaro *et al*. (2013), using fluorescent primers and an ABI3130xl genetic analyzer (Applied Biosystems, Foster City, CA), with some minor modifications for 11 SSR markers. The remaining two (VVMD25 and VVMD32) were analyzed separately, as single markers. SSR allele calling was performed using ABI Prism GeneMapper software version 3.0,

Table 1

Accession list, general information and SSR matches

Accession no.	DNA no.	Accession name	Type of flower (1)	Berry skin colour	Used for (2)	Year of ampelographic description by Constantinescu *et al.*	Biological status of accession (3)	Presence in collections (4)	Matches by SSR profile	VIVC no.	VIVC prime name
ROM045-003	180.12.R	Alb românesc (misnomer)	H	green	W	1961	360	2, 3	Sarba	10738	Sarba
ROM051-237	178.12.R	Ardeleancă	H	green	W	1959	360	2, 3	Bakator belyi	904	Bakator belyi
ROM06-0011	163.12.R	Bacator	FFr	rose	W	1959	360	1, 3	Bakator roz	905	Bakator roz
ROM051-238	161.12.R	Băbească neagră	H	blue black	W	1959	320	1, 2, 3	Babeasca neagra	843	Babeasca negra
ROM051-239	203.12.R	Băşicată	H	green	W	1961	360	2, 3	Basicata	1022	BASICATA
ROM045-025	206.12.R	Bătută neagră	H	blue black	W/T	1959	360	2	Batuta neagra	1042	Batuta neagra
ROM045-172	208.12.R	Negru românesc		black	W	1966	360	2, 3			
ROM051-240	212.12.R	Berbecel	H	green	W	1959	360	2, 3	Berbecel	1148	Berbecel
ROM045-037	181.12.R	Braghină albă*	FFr	green	W	-	360	2, 3		1645	Braghina alba
ROM045-036	182.12.R	Braghină roz	FFr	rose	W	1959	360	2	Braghina rosie	1644	Braghina rosie
ROM06-0024	157.12.R	Busuioacă de Bohotin	H	red	W	1960	320	1, 3	Muscat à petits grains blancs (somatic variant)	8248	Muscat à petits grains
ROM06-0026	160.12.R	Cadarcă	H	black	W	1959	320	1, 3	Kadarka kek	5898	Kadarka kek
ROM045-048	184.12.R	Cârcioasă	FFr	green	W	1965	360	2, 3	Balint weiss	935	Balint weiss

Tab. 1, continued

ROM051-241	185.12.R	Crâmpoşie	FFe	green	W/T	1959	330	2, 3	Crimposie	3237	Crimposie
ROM045-051	201.12.R	Ceauş alb	FFr	green	T	1959	360	2, 3	Chaouch blanc	10196	Chaouch blanc
ROM051-242	202.12.R	Ceauş roz	FFe	rose	T	1959	360	2, 3	Chaouch rozovyi	2507	Chaouch rozovyi
ROM051-243	183.12.R	Cioinic	FFe	green	W/T	1961	360	2, 3	Cioinic	2674	Cionic
ROM051-246	177.12.R	Coarnă albă	FFr	green	W/T	1959	360	1, 3	Coarna alba	2724	Coarna alba
ROM051-248	204.12.R	Coarnă roşie	FFr	red	T	1961	360	2, 3	Coarna rosie	2728	Coarna rosie
ROM051-247	176.12.R	Coarnă neagră	FFr	black-red	T	1959	320	1, 2, 3	Coarna neagra	2726	Coarna neagra
ROM051-244	213.12.R	Coada oilor/Ovis	H	green	W	1962	360	2, 3	Juhfark	5852	Juhfark
ROM06-0046	169.12.R	Creaţă	H	green	W/T	1959	360	1, 3	Kreaca	6501	Kreaca
ROM051-249	200.12.R	Creaţă de Banat				1959	360	2, 3			
ROM051-250	187.12.R	Cruciuliţă	H	green	W	1961	360	2, 3	Cruciulita	3267	Cruciulita
ROM051-251	162.12.R	Fetească albă	H	green	W	1959	310	1, 2, 3	Feteasca alba	4119	Feteasca alba
ROM051-252	158.12.R	Fetească neagră	H	black	W	1959	310	1, 2, 3	Feteasca neagra	4120	Feteasca neagra
ROM051-253	165.12.R	Fetească regală	H	green	W	1959	310	1, 2, 3	Feteasca regala	4121	Feteasca regala
ROM051-25458	172.12.R	Frâncuşă	H	green	W	1959	320	1, 2, 3	Francuse	4221	Francuse
ROM051-255	175.12.R	Galbenă de Odobeşti (NRDIBH)	H	green	W	1959	320	1, 2, 3	Galbena de Odobesti	12727	Galbena de Odobesti
	758.16	Galbenă de Odobeşti (UASVM)									
	756.16	Galbenă de Odobeşti (RDSVO)									
	174.12.R	Zghihară de Huşi (NRDIBH)									
	759.16	Zghihară de Huşi (UASVM)				1960	320	1, 2, 3			
ROM051-274	757.16	Zghihară de Huşi (RDSVO)									
ROM045-100	186.12.R	Galbenă măruntă	H	green	W	-	360	2	Kakotrygis	5920	Kakotrygis
ROM051-256	188.12.R	Galbenă uriaşă	H	green	W	1961	360	2, 3	Galbena uriasa	4322	Galbena uriasa
ROM045-106	189.12.R	Gordan	H	green	W	1961	360	2, 3	Iordan	5544	Iordan
ROM06-0069	168.12.R	Iordanā		green		1960	320	1, 3			
ROM045-253	198.12.R	Zemoasă		green		1962	360	2, 3			
ROM051-258	190.12.R	Gordin	H	green	W	1959	360	2, 3	Gordin	4901	Gordin
ROM06-0065	166.12.R	Grasă de Cotnari	H	green	W	1959	310	1, 3	Grasa de Cotnari	4948	Grasa de Cotnari
	191.12.R	Lampău (misnomer)	H	green	W	-	360	2	Tompa Mihaly	12564	Tompa Mihali
ROM051-259	170.12.R	Majarcă albă	H	green-rose	W	1960	360	1, 3	Slankamenka bela	11866	Slankamenka bela
ROM045-146	207.12.R	Moroştină*	H	green	W	-	360	2, 3	Morostina	8007	Morostina
ROM051-260	167.12.R	Mustoasă de Măderat (misnomer)	H	green	W	1960	360	2, 3		42198	Mustoasa de Maderat (not identified)
ROM051-261	192.12.R	Negru mare (questionable)*	FFe	black	W/T	1962	360	2, 3		42199	Negru mare (questionable)
ROM051-262	193.12.R	Negru moale	H	black	W	1960	360	2, 3	Negru moale	8464	Negru moale
ROM051-263	194.12.R	Negru vârtos	H	black	W	1960	360	2, 3	Mavrud Varnenskii	7540	Mavrud Varnenskii
	159.12.R	Negru vârtos (questionable)	H	black	W	-	360	1		42197	Negru vartos (questionable)
ROM045-179	195.12.R	Om rău*	H	green	W	1962	360	2, 3		8765	Om rau
ROM051-265	215.12.R	Pârciu	H	green	W	1962	360	2, 3	Pirciu	9300	Pirciu
ROM06-0103	173.12.R	Plăvaie	H	green	W	1960	330	1, 3	Plavay	9553	Plavay
	179.12.R	Alb rotund		green	W	-	360	2			
ROM045-206	196.12.R	Românie*	H	green	W	-	360	2, 3	Romanie§	10177	Romanie
ROM06-0134	164.12.R	Tămâioasă românească	H	green	W	1960	310	1, 2, 3	Tamaiosa rominesca (faux)	25546	Tamaioasa bucuresti
ROM045-234	214.12.R	Tâţa caprei albă	H	green	T	1962	360	2	Tsitsa kaprei	16449	Tsitsa kaprei
ROM051-268	210.12.R	Tâţa caprei neagră	H	black	T	-	360	2, 3	Hora	5423	Hora
ROM051-269	211.12.R	Tâţa vacii albă	FFe	green	T	1962	360	2, 3	Halholyag	6419	Halholyag
ROM045-237	209.12.R	Tâţa vacii neagră*	H	black	T	-	360	2	Kozi Cici cherveni	25547	Kozi Cici cherveni
ROM045-238	199.12.R	Teişor (misnomer)	H	green	W	1959	360	2, 3	Ezerjo	4027	Ezerjo
ROM051-272	197.12.R	Vulpea	H	black	W/T	1962	360	2, 3	Vulpea	13186	Vulpea

(1) H = hermaphrodite; FFr = female functionally with reflexed stamens; FFe = female funcionally with erect stamens.
(2) W = wine, T = table.
(3) 310 = local cultivar, spread all over, international cultivar; 320 = major local cultivar, of local importance, but extensively grown; 330 = minor local cultivar, of local importance, fairly utilized; 360 = local neglected cultivar, at risk of extinction.
(4) 1 = UASVM, 2 = RDSVO, 3 = NRDIBH; *genotype not present in Vassal.
§ in agreement with Zulj *et al*. 2013.

with a home-made bin set produced with reference varieties. Preliminary indications about cultivar identity were obtained by comparison of the genetic profiles with literature data, *V*IVC (*Vitis* International Variety Catalogue, http://www.vivc.de/), CREA-Viticulture and Enology molecular database, partially published in the Italian Grapevine Catalogue, http://catalogoviti.politicheagricole.it/ and in the Italian *Vitis* Database, http://www.vitisdb.it/, and with the INRA molecular database of the Vassal collection (Laucou *et al*. 2011).

Statistics: Cervus software (Kalinowski *et al*. 2007) version 3.0 (http://cervus.software.informer.com/3.0/) was used for preliminary indications on possible parents-progeny trios and first degree relationships.

Results and Discussion

The 61 accessions analysed in the study belonged to 51 different genotypes. This was confirmed by both microsatellite markers and ampelography. Precise morphological descriptions of the Romanian grape germplasm, literature references, ancient records in grapevine collections and the availability of SSR-marker databases were of great assistance.

Ampelographic description: All recorded data were compared with the detailed ampelographic descriptions from old documents to obtain a preliminary confirmation of the authenticity of the studied accessions, with the exception of nine varieties: 'Galbenă măruntă', 'Lampău', 'Moroştină', 'Negru vârtos' (questionable), 'Alb rotund', 'Ţâţa caprei neagră' and 'Ţâţa vacii neagră', for which no information was found, and 'Braghină albă' and 'Românie', having only partial information (Brezeanu 1912, Gorjan and Botu 2013). The ampelographic descriptions, available in the European *Vitis* database (http://www.eu-vitis.de), revealed great differences among the studied varieties, except for a few traits, such as number of consecutive tendrils (OIV 016), specific for *V. vinifera*, intensity of flesh anthocyanin coloration in the berry (OIV 231) and formation of seeds (OIV 241). The morphological and agronomic characters of the studied accessions mostly confirmed previous ampelographic documents. Few differences, at only one level of the notes corresponding to a certain characteristic were found, such as intensity of anthocyanin coloration on prostrate hairs of the shoot tip (OIV 003), density of prostrate hairs on the shoot tip (OIV 004), colour of upper side of blade (4th leaf) (OIV 051), shape of blade of the mature leaf (OIV 067), degree of opening/overlapping of petiole sinus (OIV 079), shape of base of petiole sinus (OIV 080), density of prostrate hairs between main veins on lower side of blade (OIV 084), bunch density (OIV 204), bunch shape (OIV 208), berry shape (OIV 223), sugar content of must (OIV 505) and total acidity of must (OIV 506). These differences could be ascribed to the range of expected variability of morphological and agronomic characteristics linked to environment, cultural conditions, health status of vines, and might also depend on interpretation by ampelographers.

Table and table/wine accessions, in comparison with varieties commonly used for wine production, were characterized by larger and heavier bunches, bigger berries and juice with lower acidity and sugar content.

Among the 51 genotypes found in this study, thirteen were characterized by functionally female flowers. The percentage (25.5 %) observed in this study was a relatively high proportion taking into account the average number observed world-wide (i.e. about 8 %, Boursiquot *et al*. 1995) and highlighted the originality of the Romanian gene pool. This trait is considered as ancestral and related to *Vitis vinifera* domestication, however it could be inherited through segregation by sexual reproduction, given that cultivated grapevines frequently carry a female allele (Hf), while homozygous hermaphroditic vines (HH) are rare (Fechter *et al*. 2012). In addition, this peculiar character in grapevine has aroused the interest of breeders, because it simplifies cross hybridizations in breeding programmes (Chaïb *et al*. 2010). Morphologically, only eight of them produce flowers with well-formed ovaries, stigma, style and anthers with shorter filaments, reflexed outwards from the ovary: 'Bacator', 'Braghină roz', 'Braghină albă', 'Cârcioasă', 'Ceauş alb', 'Coarnă albă', 'Coarnă roşie', 'Coarnă neagră'. The other five develop hermaphrodite flowers with anthers inclined outwards: 'Crâmpoşie', 'Ceauş roz', 'Cioinic', 'Negru mare' and 'Ţâţa vacii albă' (Tab. 1). These flowers, with stamens sloping outwards from the ovary, are considered apparently normal, but functionally female (Constantinescu 1958). All these varieties have common characteristics: a) the ratio between length of stamens and length of pistil is ≤ 1; b) the pollen is sterile, abundant and sometimes with acorn shape ('Braghină roz', 'Ceauş roz', 'Ceauş alb', 'Coarnă albă'); c) given pollen sterility, yield is variable, depending on weather conditions during anthesis; moreover, they require male or hermaphroditic vines nearby, having the same flowering period to enable pollination. The inconstancy of grape yield was the main reason for the declining interest in these varieties. Nowadays only 'Coarnă neagră' is grown on large areas (over 100 ha) being remarkable for the special appearance and taste of grapes, and also 'Crâmpoşie' for its very good qualities as both table and wine grapes.

Many of these autochthonous varieties, expressing high sugar content and equilibrate acidity of the must, have aroused breeders' interest. Elite clones with special qualities were selected and approved for wine production, like in 'Crâmpoşie', 'Tămâioasă românească', 'Fetească albă', 'Fetească neagră', 'Fetească regală', 'Braghină roz', 'Braghină albă', 'Gordan', 'Berbecel', 'Băşicată', 'Negru moale' and 'Negru vârtos', or for table grapes with pleasant appearance and flavour, like in 'Coarnă albă', 'Coarnă neagră', 'Coarnă roşie' and 'Ţâţa caprei albă'.

Genotyping and identification: Fifty-one distinct SSR profiles were found (see Table, suppl. data). Interestingly, 'Iordanā' accession showed a slightly different SSR profile from the other two synonyms 'Gordan' and 'Zemoasă' at VVS2 locus, being heterozygous (133-143), instead of homozygous (133-133); 'Plăvaie' was triallelic at VVMD32 locus (253, 265 and 273). A preliminary indication on genotype identity was first obtained by comparison of each SSR profile with literature data, *V*IVC molecular database, CREA-Viticulture and Enology and INRA molecular

databases. On the basis of genotyping results, ampelographic comparisons were made with available descriptions and historical documents for appropriate cultivar identification. Most accessions were identified by confirming already known information. In the following we only comment on the new identifications obtained.

Updating of previously uncorrect identifications: 'Busuioacă de Bohotin' is a major local cultivar, grown on 216 ha. Different opinions exist about its origin: a) it is the result of empirical selection from wild grapevines present along the Prut River; b) it was brought from ancient Greece together with 'Tămâioasă românească'; c) its Romanian origin is proved by the connected name – the village of Bohotin. While the SSR profile of 'Busuioacă de Bohotin' matched that of 'Muscat à petits grains blancs', the red berry colour and ampelographic description showed it as being very similar to 'Muscat à petits grains rouges' (*V*IVC 8248). For these reasons we concluded that 'Busuioacă de Bohotin' is the red somatic variant for berry colour of 'Muscat à petits grains blancs' and that it does not correspond to 'Muscat rouge de Madere', as previously stated by Constantinescu *et al*. (1960). The same authors and the "International List of Vine Varieties and their Synonyms" (OIV 2013) considered 'Tămâioasă românească', one of the most important and appreciated grapevine varieties in Romania, to be identical to 'Muscat à petits grains blancs'. However, in this study it turned out that the analyzed accession (ROM06-0134) did not share the SSR profile of 'Muscat à petits grains blancs', but matched the 'Tamaiosa rominesca' accession FRA139-0Mtp1091, grown in Vassal and introduced from the Bucharest collection in 1961. After genotyping and owing to the information given by Constantinescu *et al*. (1960), the French accession was considered a misnomer and renamed as 'Tamaiosa rominesca faux #3426' (faux means wrong in French). Ampelographic distinction of this genotype and 'Muscat à petits grains blancs' is evident, despite their close genetic relationship because present molecular data and Lacombe *et al.* (2013) showed that this genotype is most likely a 'Muscat à petits grains blancs' offspring (Tab. 2). Two further 'Tămâiosă' accessions entered Vassal, one from Bulgaria (1950) and another from Romania (1954) and were identified as 'Muscat à petits grains blancs'. In addition, 'Tămâioasă românească' from Craiova University grapevine collection, genotyped by Žulj Mihalević *et al*. (2013), matched the 'Muscat à petits grains blancs' genetic profile, leading to the assumption that 'Tămâioasă românească' perfectly described by Constantinescu *et al.* in 1960 is really 'Muscat à petits grains blancs'. Moreover a handwritten record by the ampelographer Paul Truel of Vassal (Figure) tells that when the 'Tamaiosa rominesca' accession entered the collection three plants were 'Muscat à petits grains blancs' with strong muscat flavour and two were different with slight muscat flavour and of unknown identity, pointing to a mixture of the two varieties. The latter being kept under the accession ID FRA139-0Mtp1091. As a result of these findings a new name for 'Tamaiosa rominesca faux #3426' was proposed: 'Tamaioasa Bucureşti'. For the future a suggestion is to study the mix of varieties in old 'Muscat à petits grains blancs' vineyards all over the country to clarify the situation and thus be of benefit for Romanian viticulture.

Table 2

Possible first degree relationships inside Romanian varieties and with 'Heunisch weiss' comparing 13 SSRs without mismatching loci, computed with Cervus software and ordered by descending pair LOD scores

Putative first degree related varieties		Pair LOD score	Results of comparison with Vassal data
Coarna neagra	Hora	1,14E+15	yes
Coarna rosie	Tsitsa kaprei	9,90E+14	yes
Muscat à petits grains blancs	Tamaíoasa bucuresti	9,47E+14	yes
Balint weiss	Galbena uriasa	7,61E+14	yes
Iordan	Crimposie	7,36E+14	yes
Coarna alba	Kreaca	7,36E+14	yes
Gordin	Braghina rosie	7,24E+14	discarded by additional SSRs
Chaouch blanc	Chaouch rozovyi	7,14E+14	yes
Iordan	Heunisch weiss	7,11E+14	yes
Iordan	Plavay	6,99E+14	yes
Coarna alba	Gordin	6,84E+14	yes
Berbecel	Pirciu	6,64E+14	possible
Negru moale	Heunisch weiss	6,51E+14	yes
Bakator belyi	Bakator roz	6,31E+14	yes
Braghina alba	Braghina rosie	5,77E+14	
Morostina	Heunisch weiss	5,56E+14	yes
Balint weiss	Slankamenka bela	5,35E+14	yes
Juhfark	Romanie	5,18E+14	
Bakator belyi	Negru moale	4,93E+14	discarded by additional SSRs
Francuse	Basicata	3,85E+14	discarded by additional SSRs
Francuse	Heunisch weiss	3,07E+14	yes
Grasa de Cotnari	Heunisch weiss	2,88E+14	yes

'Galbenă uriaşă' was considered an autochthonous variety and clonal variant of 'Galbenă de Odobeşti' (Nicoleanu 1900). It has been grown here and there in Moldavia and Transilvania regions and today is maintained in two grapevine collections. 'Galbenă uriaşă' SSR profile matched that of 'Mirkovaca', an endangered Croatian variety described by Maletić *et al*. (1999 and 2015), but was different from that of 'Galbenă de Odobeşti', therefore being another variety. A first degree relationship between them can also be excluded.

The 'Om rău' accession analysed here is certainly the one described by Constantinescu *et al*. (1962) and is different from the one genotyped by Žulj Mihalević *et al*. (2013).

Varieties absent in the Romanian ampelographic literature: 'Galbenă măruntă' is one of the varieties in danger of extinction and is today main-

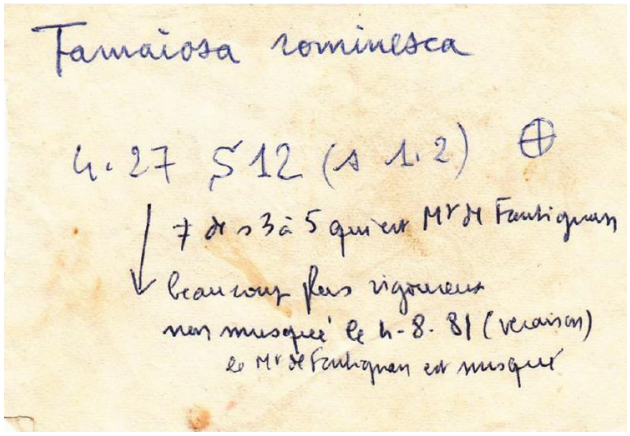

Figure: Handwritten record by Paul Truel (1924-2014), former ampelographer at the INRA Vassal collection, concerning the accession 'Tamaiosa romanesca #0Mtp1091', which means: "In plot 4.27S12, plants 1 and 2 are different from plants 3 to 5. Plants 3 to 5 are 'Muscat de Frontignan' [*i.e.* 'Muscat à petits grains blancs']. Plants 1 and 2 are much more vigorous, without muscat flavour on 4 August 1981 (véraison stage) whereas 'Muscat de Frontignan' has muscat flavour".

tained in only two grapevine collections. No documents were available about its origin or descriptions for comparison. Its SSR profile matched that of 'Kakotrygis', a Greek variety. Preliminary ampelographic observations and comparisons performed on the mature leaf and bunch showed that 'Galbenă măruntă' is very similar to the 'Kakotrygis' described by Kotinis (1984). 'Moroştină' is another very rare variety in Romanian vineyards, and no reference documents were available for description. 'Ţâţa caprei neagră' SSR profile matched that of a better known Bulgarian variety named 'Hora' and 'Tâţa vacii neagră' matched that of 'Kozi Cici cherveni', a variety grown at the Bulgarian Institut de Viticulture et d'Oenologie in Pleven. Regarding 'Braghină', Constantinescu *et al*. (1959) mentioned the long standing presence of this variety with rose grapes in Romania, where there were many populations with high variability, for example showing leaves with entire or with more lobes, with star-shaped flowers, and also populations with different berry colour and size. The two accessions 'Braghină albă' and 'Braghină roz' showed two different molecular profiles so are therefore different varieties; interestingly, they could be first degree related (Tab. 2). 'Braghină albă' analyzed by Žulj Mihalević *et al*. (2013) is a distinct variety.

New synonyms: 'Bătută neagră' and 'Negru românesc' were considered different varieties and were thus described separately by Constantinescu *et al*. in 1959 and 1966, respectively. The analyzed accessions shared the same molecular profile. The ampelographic descriptions of the two cultivars from the old documents, in comparison with those performed in our collections, confirmed the molecular results and supported their synonymy.

'Cârcioasă' was described by Constantinescu *et al*. (1965) as an old autochthonous variety, rarely grown and without any synonym. However, our results showed that 'Cârcioasă' shared the same genotype with 'Balint weiss', therefore being an additional, new synonym. 'Coada oilor' was described as 'Ovis' by Constantinescu *et al*. (1962). Considered as autochthonous and grown since long before the phylloxera invasion, the cultivar is today maintained only in collections. Its SSR profile matched that of 'Juhfark' (Galbacs *et al.* 2009, Jahnke *et al*. 2009). Comparison of ampelographic features of 'Juhfark' and 'Ovis' as given in the literature (Nemeth 1970, Constantinescu *et al*. 1962) and 'Juhfark' and 'Ovis' accessions described by Geilweilerhof and Ştefăneşti respectively likewise attested to their identity.

'Gordan', 'Iordanā' and 'Zemoasă' accessions showed identical microsatellite profiles, proving to be synonyms. All these varieties are considered autochthonous and have been grown since before the phylloxera invasion. Constantinescu *et al*. in 1960 and 1961 mentioned the synonymy between 'Gordan' and 'Iordan' (in Romanian literature present as 'Iordanā'), their names having a common origin from *Iordan*, becoming *Giordan* and after that *Gordan*. Our results are in accordance with findings in the French Vassal collection (https://bioweb.supagro.inra.fr/collections_vigne) and in the European *Vitis* Database (http://www.eu-vitis.de). Today, 'Iordanā' is a major cultivated variety grown mostly in western and central Romania, 'Gordan' is grown in a small area especially in the southeastern wine region and 'Zemoasă' is only rarely present in private vineyards or in repositories. The SSR profiles of 'Gordin' and 'Gordan' proved to be different, excluding the possibility of being synonyms, unlike the Žulj Mihalević *et al*. (2013) findings and supporting the old descriptions (Constantinescu *et al.* 1959 and 1961).

Interesting results were obtained with two major cultivars of local importance, extensively grown in Romania, 'Zghiharā de Huşi' and 'Galbenă de Odobeşti', nowadays considered to be different varieties. Constantinescu (1958) wrote that "'Galbenă de Odobeşti' is one of the old Romanian varieties with a large growing area in vineyards of Odobeşti and Panciu; it resembles 'Bătută neagră', but has a different grape colour and is a synonym of 'Zghiharā de Huşi'". Even though Constantinescu *et al.* described 'Galbenă de Odobeşti' and 'Zghiharā de Huşi' separately in 1959 and 1960 respectively, our SSR data confirmed this synonymy, but exclude a parent-offspring relationship between 'Galbenă de Odobeşti' and 'Bătută neagră'.

Questionable genotypes: Some accessions, being true-to-type on the basis of ampelographic descriptors, turned out to be critical after comparison of their SSR profiles with literature data. 'Negru mare' was classified as questionable, because it differs from the one grown in Vassal. Further observations are necessary to check the authenticity of this variety with respect to the available ampelographic description. 'Negru vîrtos' (at present 'Negru vârtos') has been grown in Romania since long before the phylloxera invasion. Constantinescu (1958) mentioned two biotypes of 'Negru vîrtos', one with functionally female flowers and another with hermaphrodite flowers, the latter being morphologically very similar to 'Mavrud Varnenski'. The genetic profile of our 'Negru vârtos' accession (ROM051-263) matched that of 'Mavrud Varnenski', confirming the old information. Two years later Constantinescu *et al*. (1960) wrote that there are many 'Negru vîrtos' biotypes. So we

hypothesized that 'Negru vârtos' could represent a larger group of homonyms, encompassing 'Mavrud varnenski', the female genotype grown in Vassal (turned out to be true-to-type) and this third variety (159.12.R) genotyped in the present research.

Misnomers: 'Alb românesc' (ROM045-003) was shown to be a misnomer as the SSR profile and ampelographic traits both match 'Sarba', a new bred Romanian cultivar. 'Teişor' (ROM045-238) was regarded as a misnomer expected to be a synonym of 'Harslevelu' (Constantinescu *et al*. 1959), and not 'Ezerjo'. In fact, 'Teişor' is the diminutive of 'Tei', meaning lime, and many synonyms of 'Harslevelu' have names referring to the shape of its leaf, similar to that of the lime tree, like 'Lindenblättriger', 'Feuille de Tilleul', 'Frunzā de Tei'. 'Mustoasā de Māderat' did not match the true-to-type grown in Vassal; our genotype is original and no matches were found with other already genotyped varieties. So it remained anonymous. 'Lampāu' was another misnomer matching 'Tompa Mihaly' SSR profile and the ampelographic description of this accession also did not correspond to literature data.

Possible trios for parents and offsprings and first degree relationships: Possible parents-offspring trios and first degree relationships were found. Cervus software indicated two possible trios without mismatchings for candidate parents and progeny. The first one was that 'Feteasca regala' could be the progeny of 'Feteasca alba' and 'Francuse', with a LOD score of 1.49 E+15, supporting with 6 additional SSRs what Lacombe *et al*. (2013) already found using 20 SSRs. This information was partially different from the genitors supposed by Constantinescu *et al*. (1959), *i.e.* 'Feteasca alba' and 'Grasa de Cotnari', this latter variety being excluded with certainty by molecular data. The second trio indicated 'Braghina alba' as the progeny of 'Coarna alba' x 'Galbena de Odobesti', 'Coarna alba' being the putative mother given the functionally female sex of flowers and 'Galbena de Odobesti' the possible father with hermaphrodite flowers. In this case the LOD score was a little bit higher: 1.71 E+15. Cervus software indicated a list of possible first degree relationships (PO), reported in Tab. 2. Some of them were partial parentages of already completed trios, such as 'Coarna rosie' and 'Tsitsa kaprei', full parentage being 'Coarna rosie' = 'Tsitsa kaprei' and 'Parmak crven'; 'Crimposie' and 'Iordan', full parentage being 'Crimposie' = 'Iordan' and 'Beala Debela'; 'Balint weiss' and 'Slankamenka bela', full parentage being 'Slankamenka bela' = 'Balint weiss' and 'Razachie rosie' (Lacombe *et al*. 2013). The other PO relationships were evaluated by comparison with the larger INRA database, apart from two, because the varieties involved, 'Braghina alba' and 'Romanie', were not present in Vassal. The preliminary PO indications were mostly confirmed, but three cases discarded by additional markers. Five cultivars, namely 'Francuse', 'Grasa de Cotnari', 'Iordan', 'Morostina' and 'Negru moale' showed a PO relationship with 'Heunisch weiss'/'Gouais blanc'. So, 'Heunisch weiss'/'Gouais blanc', one of the most prolific founders of the present grapevine assortment (Maul *et al*. 2015) seems also to have played an important role in the birth of some Romanian cultivars.

Conclusions

Both morphological descriptors and SSR markers proved to be efficient at confirming or detecting synonyms, homonyms, questionables, misnomers and unique genotypes in Romanian grapevine germplasm collections, therefore helping to update the information about the grapevine germplasm preserved there. Together, these two methods also clarified some previous suppositions about the origin of local/autochthonous varieties and brought out new aspects to be analyzed.

Ampelographic methods used to characterize varieties through standardized description, based on phenotypic traits, were applied to obtain an up-to-date description of the studied accessions. These ampelographic descriptions together with genetic profiles and photos are available via the European *Vitis* Database. They represent a reference for the authenticity of the studied accessions and their respective varieties. A wide range of variability was determined among the studied cultivars regarding certain morphological and agronomic characters, which are especially valuable for the autochthonous grape varieties in danger of extinction.

The molecular data complemented conventional descriptions, succeeding in identifying almost all the analyzed accessions, improving the knowledge on Romanian grapevine varieties. The accurate identification obtained allowed the provenance of some varieties previously considered as autochthonous to be known and, viceversa, to detect autochthonous varieties previously considered as imported. The pedigrees of some of them were confirmed and additional information was produced about possible PO relationships, delegated to future research.

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