Ciência Téc. Vitiv. 30(1) 43-52. 2015

IDENTITY, SYNONYMIES AND HOMONYNIES OF MINOR GRAPEVINE CULTIVARS MAINTAINED IN THE PORTUGUESE AMPELOGRAPHIC COLLECTION

IDENTIFICAÇÃO, SINONÍMIAS E HOMONÍMIAS DE VARIEDADES MINORITÁRIAS PRESERVADAS NA COLEÇÃO AMPELOGRÁFICA PORTUGUESA

Antonios Alifragkis[1,2,7], Jorge Cunha[3,4,7*], Joana Pereira[5], Pedro Fevereiro[4,6], José E. J. Eiras Dias[3]

[1] Instituto Superior de Agronomia, Tapada da Ajuda, Lisboa, Portugal.

[2] Aristotle University of Thessaloniki, Greece

[3] Instituto Nacional de Investigação Agrária e Veterinária, Quinta d'Almoinha, Dois Portos, Portugal.

[4] Universidade Nova de Lisboa, Instituto de Tecnologia Química e Biológica (ITQB), Green-it Unit, Portugal

[5] Instituto Superior de Engenharia, Campus da Penha, Faro, Portugal.

[6] Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Vegetal, Portugal

[7] both authors contributed equally to this work.

*corresponding author: Tel: +351261712106, e-mail: jorge.cunha@iniav.pt

(Received 29.01.2015. Accepted 20.07.2015)

SUMMARY

Nineteen SSR were used to genotype twenty eight accessions from Portugal, France, Greece, and Spain, preserved at the Portuguese National Ampelographic Collection (international code - PRT 051). Some of these accessions have never been genotyped and are minor, underused cultivars. Eight cases of synonymies and one case of homonymy were detected. The identification of synonyms in underused cultivars increases de possibility of obtaining adequate plant material for certification purposes and commercialization. The new unique genotypes identified in this work will be proposed for inclusion in the list of authorized cultivars for wine production in Portugal. No certified plant materials exist for these cultivars.

RESUMO

Dezanove SSR foram utilizados para genotipagem de vinte e oito acessos de Portugal, França, Grécia e Espanha, preservados na Coleção Ampelográfica Nacional (código internacional - PRT 051). Algumas destas cultivares nunca foram genotipadas e são cultivares minoritárias, subutilizadas. Oito casos de sinonímia e um caso de homonímia foram identificados. A identificação de sinonímias em cultivares subutilizadas aumenta a possibilidade de encontrar material vegetal adequado para fins de certificação e comercialização. Os novos genótipos únicos identificados neste trabalho serão propostos para inclusão na lista de cultivares autorizadas para produção de vinho em Portugal. Estes novos genótipos não possuem material vegetal certificado para propagação.

Key words: Minor Portuguese cultivars, genotyping, synonymies and homonymies, SSR.
Palavras-chave: Cultivares Portuguesas minoritárias, genotipagem, sinónimos e homónimos, SSR.

INTRODUCTION

High genetic variability allied to a long history and widespread area of cultivation, natural selection, natural crossing between cultivars, breeding and selection programs and the adaptation of cultivars to new environments, all are reasons for the precious and almost endless grapevine genetic diversity at our disposal. Viala and Vermorel, (1901-10) reported more than 24000 denominations of *Vitis vinifera* L, believed to correspond to about 8000 – 9000 concrete cultivars. In this context a meticulous identification and classification of the different cultivars is a difficult process. Synonyms (different names

Article available at http://www.ctv-jve-journal.org or http://dx.doi.org/10.1051/ctv/20153001043

attributed to the same cultivar in different cultivation areas) and homonyms (attribution of the same name to different cultivars) found in different wine regions cause great difficulties in cultivar management. In Portugal, several synonyms were confirmed by Lopes *et al.*, (1999, 2006), namely: i) cultivar groups of 'Fernão Pires', from three geographic origins are identical to 'Maria Gomes'; ii) 'Castelão' cultivar is the same which 'Periquita', 'Castelão Francês', 'João Santarém' and 'Trincadeira'; III) 'Moscatel de Setúbal' and 'Muscat of Alexandria' are also synonyms, IV) 'Malvasia Fina' cultivar is the same as 'Assario', 'Boal' from Madeira Island and 'Boal Cachudo', V) 'Sercial' grown in Madeira island is the same which 'Esgana Cão', VI) 'Gouveio' and 'Gouveio Roxo' showed the same allelic profile as 'Verdelho' grown in the Dão region, and VII) 'Verdelho' from Island Madeira and Azores are also synonyms.

An Iberian example of synonyms is the cultivar 'Aragonez', known as 'Tinta Roriz' in the Douro and 'Tempranillo' in Spain (Eiras Dias *et al.*, 2011; Ibáñez *et al.*, 2012). Another example is the cultivar 'Grenache' in France, known as 'Garnacha Tinta' in Spain, 'Cannonau' in Sardinia and 'Red Tokai' in the Italian peninsula. Many other examples may be found all over the world (Calo *et al.*, 1990). Grando *et al.*, (2000) identified 11 cases of synonyms within cultivars grown in the Trentino area in Northern Italy using 7 microsatellite (SSR) loci. Cases of false synonymy or homonymy may also be found in many historically grapevine growing areas. An example is the variety 'Limnio' also known as 'Limniona', cultivated mainly in the Greek Aegean islands that differs from the variety 'Limniona' also known as 'Limnio' mainly cultivated in Thessaly (Stavrakas, 2011).

The correct identification of the different cultivars is important in order to optimize cultivation practices and wine authenticity. The traditional ampelographic methods tend to be inconvenient as they require an expert in classic ampelography (morphologic and ampelometric identification in field conditions) able to perform a rigorous monitoring during vegetative growth cycle. However, these phenotypic characteristics are often influenced by nutritional and phytosanitary status and by environmental conditions. These reasons led to the development and use of molecular methods for cultivar identification and certification. The International Organization of Vine and Wine (OIV) suggested the use of 6 SSR loci as a reference for the genetic identity of cultivars (OIV, 2009).

In this study a set of 19 microsatellite loci were used, which includes only four of the OIV above mentioned along with other 15 previously described in Laucou *at al.* (2011), which are very polymorphic and are scattered in eighteen out of nineteen linkage groups in Vitis. The discriminatory ability of these 19 SSR markers will be used in the present work to differentiate grapevine cultivars and/or clones, and will be used in the future on phylogeny and genetic diversity studies. The objective of this study was to genotype minor cultivars from Portugal (19 accessions), as well as, accessions collected in France (4), Spain (4) and Greece (1), kept at the Portuguese National Ampelographic Collection (CAN, international code PRT051). Some of these accessions have never been genotyped, given that they are local and underused cultivars, not used today in the wine industry. Identifying synonyms in minority cultivars increases the possibility of obtaining adequate plant material for certification purposes and for commercialization.

MATERIAL AND METHODS

Plant Material

Cultivars to be genotyped are maintained in the Portuguese Ampelographic Collection (CAN) for the last 20 years and were originally collected in germplasm banks from France, Spain and Portugal. CAN was established in 1988 at INIAV-Dois Portos, 60 Km north of Lisbon and is the international reference for the Vitis genus in Portugal (reference - PRT 051).

The twenty eight accessions used in this study are listed in Table I. Several of the Portuguese cultivars are known to be old cultivars, close to extinction, with some of them kept only at CAN. These cultivars were introduced in the collection as a result of intense prospection in all the Portuguese wine regions, including the Azores and Madeira insular regions. The Spanish (4), French (4) and Greek (1) cultivars were used in this study because some of them have Portuguese names and/or because they show morphologic similarities to Portuguese cultivars (see in Table I and Supplemental Table I, groups of cultivars that previous morphologic data suggest the existence of synonyms). The cultivars Riesling, Touriga Nacional and Cinsaut were used as controls while inter-specific hybrid Barco do Porto and Vitis aestivalis were used as out-groups (Table I).

When possible, cultivars are identified according the prime name and variety number (Table II) proposed by the Vitis International Variety Catalogue (VIVC, www.vivc.de).

Table I

List of plant material used in this study

Lista do material vegetal utilizado no presente estudo

Country of origin[a] (Institute code)	Accession name (accession number) in PRT051	Color[b]	Acronym cultivar	Groups of [c] preliminary synonyms
France (FRA139)	Cinsaut (53805)	N	Cinsaut	II
France (FRA139)	Riesling (53209)	B	Riesl	IV
France (FRA139)	Santa Paula (53210)	B	SantPaula	III
France (FRA139)	Vitis Aestivalis (50417)		V_Aest	I
Greece (nursery)	Limnio	N	Limnio	
Portugal (PRT078)	Alvarelhão Ceitão (41209)	N	Alvar_Ceit	XII
Portugal (CVRVV)	Azal Espanhol (41201)	N	AzalEspa	X
Portugal (CVRVV)	Barco do Porto (50402)		Bar_Por	I
Portugal (PRT051)	Boal Espinho (52017)	B	Boal_espi	IX
Portugal (PRT051)	Carignan Francês (51401)	N	Carig_Fran	II
Portugal (PRT078)	Cornichel Branco (40405)	B	CornicBran	III
Portugal (IV Madeira)	Desconhecida, Madeira (50108)	B	Desc_Mad	V
Portugal (PRT051)	Diagalves (52513)	B	Diag	VII
Portugal (Pinhel)	Formosa (50614)	B	Form	VII
Portugal (PRT078)	Gouveio (52112)	B	Gouveio	XI
Portugal (IV Madeira)	Malvasia Branca de S. Jorge (40604)	B	Malv_Banc_S_Jo	V
Portugal (PRT051)	Pintado dos Pardais (52115)	B	Pint_Pard	IX
Portugal (PRT078)	São Saul (51502)	N	Sao_Sau	II
Portugal (PRT051)	Teinturier (53807)	N	Teint	VIII
Portugal (IV Madeira)	Tinta Negra Mole (51202)	N	Tin_Neg_Mol	VI
Portugal (PRT078)	Touriga Nacional (52206)	N	TourNac	X
Portugal (CVRVV)	Transâncora (41206)	N	Trans	VIII
Portugal (CVRVV)	Verdial (41207)	N	Verd_Verd	XII
Portugal (PRT078)	Verdial (41208)	N	Verd_Dou	XII
Spain, (ESP074)	Albariño (51707)	B	Albari	IV
Spain, (Galicia, EV)	Godello (40303)	B	Godello	XI
Spain, (ESP080)	Mollar (41809)	N	Mollar	VI
Spain, (ESP074)	Verdejo Negro (51805)	N	Verd_Neg	VI

[a]Institute code: FRA139: INRA - Unité Expérimentale du Domaine de Vassal & Montpellier SupAgro; PRT078: Centro de Estudos Vitivinicolas do Douro; IV Madeira: Instituto do Vinho da Madeira; PRT051: Estaçao Vitivinicola Nacional; Pinhel: Direção Regional de Agricultura e Pescas do Centro; CVRVV: Comissão de Viticultura da Região dos Vinhos Verdes; Galicia, EV: Estación Vitivinícola y Enológica de Galicia; ESP080: Instituto Madrileño de Investigación Agraria y Alimentaria (I.M.I.A); ESP074: Junta de Andalucia; [b] Color of berry skin B - white berry; N - black berry; [C] Groups of preliminary synonyms based only in morphologic data (results in Supplemental Table 1).

DNA Extraction and PCR Amplification

Genomic DNA was isolated from young leaves according to the protocol suggested by Lodhi *et al.*, (1994) in order to minimize the effect of contaminants such as polyphenols and polysaccharides. Harvesting yields of DNA ranged from 30 to 150 ng/µL and all samples were diluted to an adequate concentration (10 to 20 ng/µL). Nineteen nuclear microsatellites were divided into 6 multiplex mixes (Mplex1 SSRs:

VVMD27, VVMD25, VVMD28 and VVMD32; Mplex2 SSRs: VVMD5, VVMD7 and VVS2; Mplex3 SSRs: VMC4f3 and VVMD24; Mplex4 SSRs: VVIb01, VVIn73, VVIh54 and VVIq52; Mplex5 SSRs: VVIp31, VVIp60 and VVMD21; Mplex6 SSRs: VMC1b11, VVIn16 and VVIv67. One primer of each pair was previously labeled with fluorescent Dye (blue or green or black). Each multiplex reaction was prepared according to the manufacturer's instructions of Maxima Hot Start PCR Master Mix (2x).

The Biometra T-Gradient Thermoblock thermocycler was programmed to 15 min at 94 ºC, followed by 30 sec at 94 ºC, 90 sec at 56 ºC, 60 sec at 72 ºC and a final extension step of 30 min at 72 ºC. After verifying the PCR products using gel electrophoresis, capillary electrophoresis was carried out in the automatic sequencer CEQ 8000 Genetic Analysis System (Beckman Coulter). DNA size standard-400 (P/N 608109) was included as an internal sizing standard and labeled products were analysed and sized using the CEQ System (version 9) software.

Table II

Genetic profiles of 28 accessions using 19 microsatelite loci. Allele size is given in base pairs.

Perfis genéticos dos 28 acessos, utilizando 19 loci de microssatélites. Os tamanhos dos alelos são apresentados em pares de bases.

Accession number [a)]	Prime name [b)]	Portuguese Legal name [c)]	VMC1b11	VMC4f3	VVIb01	VVIh54	VVIn16	VVIn73	VVIp31
41209	Alvarelhão Ceitão	Alvarelhão-Ceitão	171:183	165:171	288:288	163:167	137:151	263:263	188:190
50402	Barco do Porto	Barco do Porto	167:167	163:171	290:295	163:163	149:159	261:261	178:192
52115	Boal Espinho	Boal-Espinho	165:183	165:205	288:288	163:163	151:151	263:263	188:188
52017	Boal Espinho	Boal-Espinho	165:183	165:205	288:288	163:163	151:151	263:263	188:188
51401	Cinsaut	Cinsaut	165:169	171:187	290:294	163:167	149:149	263:263	180:186
51502	Cinsaut	Cinsaut	165:169	171:187	290:294	163:167	149:149	263:263	180:186
53805	Cinsaut	Cinsaut	165:169	171:187	290:294	163:167	149:149	263:263	180:186
40405	Cornichel Branco	Cornichel-Branco	184:184	185:187	290:294	165:177	149:151	263:263	178:188
53210	Cornichel Branco	Cornichel-Branco	184:184	185:187	290:294	165:177	149:151	263:263	178:188
50614	Mantuo	Diagalves	184:188	185:185	290:290	165:165	151:151	263:263	174:178
52513	Mantuo	Diagalves	184:188	185:185	290:290	165:165	151:151	263:263	174:178
40303	Gouveio	Gouveio	165:169	177:185	288:290	163:165	149:151	261:263	178:188
52112	Gouveio	Gouveio	165:169	177:185	288:290	163:165	149:151	261:263	178:188
	Limnio		183:183	207:225	294:294	165:165	149:151	255:263	176:188
50108	Malvasia Branca de Sao Jorge	Malvasia -de -São -Jorge	183:187	181:187	290:299	163:167	151:151	263:263	186:190
40604	Malvasia Branca de Sao Jorge	Malvasia -de -São -Jorge	183:187	181:187	290:299	163:167	151:151	263:263	186:190
41809	Mollar Cano	Negra -Mole	184:188	164:~	290:290	167:167	149:151	263:263	174:190
53209	Riesling Weiss	Riesling	165:184	171:187	288:298	149:163	149:151	263:263	174:182
51707	Savagnin Blanc	Savagnin-Blanc	169:171	171:177	288:294	163:163	149:157	263:263	178:192
53807	Teinturier	Teinturier	171:183	171:185	290:295	163:163	151:151	263:263	176:192
51202	Molar	Tinta -Negra	165:171	177:205	288:294	163:167	149:151	263:263	178:188
51805	Molar	Tinta -Negra	165:171	177:205	288:294	163:167	149:151	263:263	178:188
52206	Touriga Nacional	Touriga -Nacional	167:169	177:205	294:294	165:167	149:149	263:263	182:182
41206	Transancora	Transâncora	167:171	171:171	288:295	165:165	151:151	263:263	178:188
41201	Transancora	Transâncora	167:171	171:171	288:295	165:165	151:151	263:263	178:188
41208	Verdial Tinto	Verdial-Tinto	183:187	181:205	288:288	163:163	149:151	255:263	176:188
41207		New genotype	167:183	171:177	288:295	167:167	141:151	263:263	178:178
50417	*Vitis aestivalis* Michaux Var. *aestivalis*	New genotype	171:187	165:165	292:292	165:165	149:149	263:263	174:182

Table II

Continuation.

Continuação.

Accession number[a)]	VVIp60	VVIq52	VVIv67	VVMD21	VVMD24	VVMD25	VVMD27	VVMD28	VVMD32	VVMD5	VVMD7	VVS2
41209	317:320	77:79	361:371	241:241	206:214	248:254	184:184	257:257	257:271	224:240	240:244	140:148
50402	309:320	77:77	341:341	239:247	206:214	240:248	176:184	233:251	271:271	224:238	238:248	130:130
52115	303:320	79:83	369:369	241:247	208:208	240:254	176:192	235:257	253:271	222:234	240:244	140:140
52017	303:320	79:83	369:369	241:247	208:208	240:254	176:192	235:257	253:271	222:234	240:244	140:140
51401	315:319	83:83	360:360	247:253	206:210	238:248	176:178	227:233	255:261	223:223	243:247	131:131
51502	315:319	83:83	360:360	247:253	206:210	238:248	176:178	227:233	255:261	223:223	243:247	131:131
53805	315:319	83:83	360:360	247:253	206:210	238:248	176:178	227:233	255:261	223:223	243:247	131:131
40405	315:324	79:83	353:353	253:253	206:215	238:254	178:191	243:257	255:259	223:234	249:253	131:141
53210	315:324	79:83	353:353	253:253	206:215	238:254	178:191	243:257	255:259	223:234	249:253	131:141
50614	317:324	83:83	353:368	247:247	206:206	254:254	178:182	233:259	251:271	232:236	243:253	141:149
52513	317:324	83:83	353:368	247:247	206:206	254:254	178:182	233:259	251:271	232:236	243:253	141:149
40303	317:319	79:83	363:368	247:251	212:214	248:248	182:186	233:257	251:271	223:236	239:243	149:155
52112	317:319	79:83	363:368	247:251	212:214	248:248	182:186	233:257	251:271	223:236	239:243	149:155
(Limnio)	319:330	81:81	355:355	245:255	206:206	240:240	182:186	233:265	255:271	224:226	250:252	130:142
50108	303:330	83:83	355:371	247:253	206:206	238:238	178:192	225:245	255:259	220:226	240:240	130:142
40604	303:330	83:83	355:371	247:253	206:206	238:238	178:192	225:245	255:259	220:226	240:240	130:142
41809	319:319	79:83	347:361	241:263	204:208	240:254	178:178	243:257	251:271	~:238	239:239	141:143
53209	303:322	77:83	353:360	247:247	206:214	248:254	178:186	227:233	251:271	223:232	249:257	141:149
51707	303:317	77:83	360:368	247:247	210:214	248:248	186:186	233:235	241:271	229:236	243:257	149:149
53807	315:320	79:83	361:371	245:245	210:210	240:248	178:182	235:255	253:271	224:234	240:240	144:150
51202	317:319	83:83	360:371	247:253	206:210	238:248	178:186	233:247	255:271	223:236	239:257	131:149
51805	317:319	83:83	360:371	247:253	206:210	238:248	178:186	233:247	255:271	223:236	239:257	131:149
52206	317:317	79:83	360:363	246:251	206:210	248:254	178:186	233:267	239:271	223:234	239:271	141:149
41206	313:318	77:83	365:365	241:245	204:206	238:258	186:186	257:267	241:253	222:230	240:262	134:156
41201	313:318	77:83	365:365	241:245	204:206	238:258	186:186	257:267	241:253	222:230	240:262	134:156
41208	315:324	83:83	361:371	241:247	206:208	240:252	176:180	233:251	253:253	224:226	240:240	142:142
41207	317:317	77:83	355:365	241:247	206:206	238:254	186:186	233:255	241:241	218:230	240:262	142:156
50417	319:320	77:79	361:371	245:263	206:216	238:238	178:186	227:265	249:253	236:238	236:248	120:134

a) Accession number in PRT051 collection; b) Prime name already identified in VIVC or prime name that will be proposed to VIVC. c) Portaria name already identified in Portuguese wine legislation or in bold word proposed name. ~ alleles not detected.

Phenetic relationships

The genetic distance among cultivars was calculated using the software GenAlex 6.501 (Peakall and Smouse, 2012), while MEGA 5.2 (Tamura *et al.*, 2011) was used to construct the phenetic tree based on the unweighted pair-group arithmetic average method (UPGMA) over a matrix of genetic distances obtained with the simple match algorithm. The Excel Microsatellite Toolkit software (Park, 2001) was used to identify the matches with cultivars genotyped by Lacombe *et al.* (2013).

Results obtained by Veloso *et al.* (2010) and Santiago *et al.* (2007) were also used to compare genotyped cultivars (these comparative analyses were examined only in common locus).

RESULTS AND DISCUSSION

Phenotype accessions and true genetic identity

A morphological characterization of the 28 accessions under study, based on the OIV descriptors was made (Supplemental Table I). This characterization allowed the formation of 12 groups of potential synonyms (see Table I, last column and Supplemental Table I): I) *Vitis Aestivalis*/ Barco do Porto; II) Cinsaut/São Saul/Carignan Francês; III) Santa Paula/Cornichel Branco; IV) Riesling/Albariño; V) Malvasia Branca de São Jorge/Desconhecida da Madeira; VI) Tinta Negra Mole/Mollar/Verdejo Negro; VII) Diagalves/Formosa; VIII) Teinturier/Transâncora; IX) Pintado dos Pardais/Boal Espinho; X) Azal Espanhol/Touriga Nacional; XI) Gouveio/Godello; XII) Verdial_41208/Alvarelhão Ceitão/Verdial_41207). The Greek cultivar Limnio when compared with the other accessions formed an out-group.

Amplification of 19 SSRs on the 28 accessions produced twenty different genotypes (Figure 1). Relative allele sizes were standardized using three reference cultivars (Touriga Nacional, Cinsaut and Riesling) common to the data obtained by Lacombe *et al.* (2013). Data was analyzed with Excel Microsatellite Toolkit software (Park, 2001) and ten matching genotypes were detected between the 28 accessions and the accessions genotyped by Lacombe *et al.* (2013): Touriga Nacional/Touriga Nacional (#1493); Santa Paula/Santa Paula (#701); Riesling/Riesling(#274); Cinsaut/Cinsaut (#5); Gouveio/Gouveio (Verdelho) (#296); Teinturier/Teinturier (#303); Diagalves/Diagalves (#1536); Tinta Negra Mole/Tinta Madeira (#1529); Albariño/Savagnin = Traminer (#257) and Mollar/Mollar (#1471). From these 10 matches only the CAN accession Albariño (51707), originated from ESP74 (Junta de Andalucia), is clearly misidentified in the CAN.

Historical remarks from cultivars identified as synonyms and homonyms

The genetic relationships among the 28 accessions are shown in the phenogram derived from SSR data (Figure 1). In this phenogram eight cases of synonymy and one case of homonymy were identified. The synonymous found are: i) São Saul (51502), Cinsaut (53805) and Carignan Francês (51401); ii) Tinta Negra (51202) and Verdejo Negro (51805); iii) Godello (40303) and Gouveio (52112); iv) Cornichel Branco (40405) and Santa Paula (53210); v) Formosa (50614) and Diagalves (52513); vi) Transâncora (41206) and Azal Espanhol (41201); vii) Pintado dos Pardais (52115) and Boal Espinho (52017); viii) Desconhecida da Madeira (50108) and Malvasia Branca de S. Jorge (40604).

The comparison of the genotypes show that Carignan Francês (51401) and São Saul (51502) are synonyms of Cinsaut (53805), as already expected from the comparison of the morphological descriptors recommended by OIV (OIV, 2009). The cultivar Carignan Francês (51401) was wrongly considered in Portugal as synonymy of Carignan Noir. The Vitis International Variety Catalogue does not include Carignan Francês (51401) as synonym of Cinsaut, but as synonym of Carignan Noir. ViVC should correct this synonymy and consider Carignan Francês as synonymous of Cinsaut and not of Carignan Noir.

Tinta Negra Mole (51202) or Tinta de Madeira and Verdejo Negro (51805) are synonyms (Table I and II), as showed by the morphologic descriptors of both accessions (Supplemental Table I). Comparison of our results with the VIVC (Molar, prime name and nº 15678) and with results from Ibáñez *et al.* (2003) confirmed that these cultivars are synonyms. In the Madeira Island Tinta Negra Mole is the most widely planted cultivar. In the Azores Islands Tinta Negra Mole is grown under the name of Saborinho. Tinta Negra Mole is identified as Tinta Negra in the Portuguese law that established the cultivars legally accepted to produce wine in the country (Portaria nº 380/2012 de 22 de Novembro), and, in Mainland Portugal, is found in vineyards at Colares and Pinhel under the designations of Molar and Rabo de Ovelha Tinto, respectively. Tinta Negra Mole is different from Negra Mole (52202) cultivated in the Algarve region (unpublished results). The comparison of our results with those obtained by Veloso *et al.* (2010)

shows that cultivars Mollar (41809) from Spain and Negra Mole from Algarve are synonyms. Lacombe *et al.* (2013) also identified this clear synonym (Negra Mole = Mollar_#1471) and VIVC include these two names as synonyms of Mollar Cano (VIVC nº 7901) as prime name.

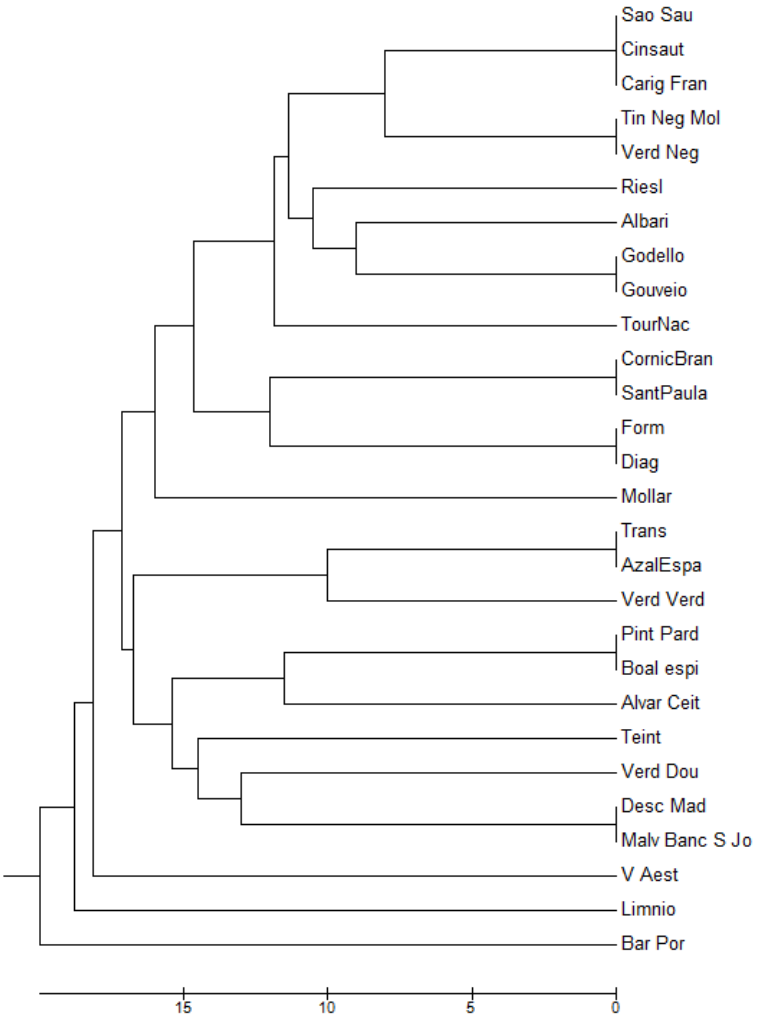

Figure 1. Phenogram of the genotyped cultivars using the simple match algorithm as coefficient of distance, and UPGMA as the clustering method.

Fenograma das cultivares genotipadas usando o algoritmo de correspondência simples como coeficiente de distância, e o método de agrupamento UPGMA

The synonymies between the cultivars Godello (40303)/Gouveio (52112); and Diagalves (52513)/Formosa (50614) have been already described on European Vitis Database (http://www.eu-vitis.de/index.php).

Cornichel Branco (40405) and Santa Paula (53210) are synonyms and neither is cited in ancient Portuguese bibliography, and thus possibly a recent genotype in the Portuguese germplasm. The VIVC database includes these two cultivars as distinct (Cornichel Branco, VIVC nº 17666 and Santa Paula, VIVC nº 23941). No new plantations are known with Cornichel Branco/Santa Paula in Portugal. In Portugal, all existing genotypes of this minor cultivar are restricted to the CAN, but are also preserved in France, INRA (FRA139).

The cultivar Transâncora (41206) and Azal Espanhol (41201) both from Vinhos Verdes provenance are new synonyms identified in this work. These cultivars are almost extinct, since no new vineyards planted

recently are known. A VIVC database refers Azal Espanhol (VIVC nº 23978) and Transâncora (VIVC nº 17734) as different cultivars without a prime name attributed. In the old Portuguese written records the cultivar Azal or Azar is the name of several black berry cultivars ("Azal", Azal Preto, Azal Azedo, Azal Fechado or Azar Hespanhol) that were different from "Traz de Âncora" (Menezes, 1900), which may be the origin of the name Transâncora. The only relations between these two cultivars are the color of the berries and the wine region of origin (Vinhos Verdes) were they were both cultivated. In the actual Portuguese legislation only the name Transâncora is mentioned.

Pintado dos Pardais (52115) / Boal Espinho (52017) are synonyms as previously proved using only morphologic data (Eiras Dias *et al.*, 2011). The VIVC does not include Pintado dos Pardais as synonym of Boal Espinho.

Desconhecida da Madeira (50108) and Malvasia Branca de S. Jorge (40604) were identified as synonyms corroborating the morphological data obtained at the CAN (Supplemental Table I). This cultivar is grown only in Madeira Island and is different from Malvasia Candida also cultivated in Madeira Island. While Malvasia Branca de S. Jorge is used in the establishment of new vineyards in Madeira Island (only standardized plant material).

The CAN accession Albariño (51707) is misidentified. The comparison of our data with the data of Lacombe *et al.* (2013), of Veloso *et al.* (2010) and of Santiago *et al.* (2007) revealed that the correct identification of this accession is Savagnin Blanc. Albariño is the Spanish cultivar synonym of the Portuguese cultivar Alvarinho (52007), native to northwest of the Iberian Peninsula abundantly grown in the Minho in Portugal and Galicia in Spain.

Verdial N (41208) cultivated in Douro and Verdial (41207) cultivated in Vinhos Verdes regions are homonymous (Figure 1, Table I). In these cases new surveys will be needed to clarity their situation to be able to assign appropriate names to each of these cultivars.

Others homonymies between the cultivars: i) Azal Espanhol (41201) and Azal (VIVC nº 815 that correspond to 52809 of CAN), ii) Alvarelhão Ceitão (41209) and Alvarelhão (VIVC nº 1650 that correspond to 53207), iii) Tinta Negra (51202) and Negra Mole (prime name Mollar Cano, VIVC nº 7901 and correspond to 52202 of CAN), iv) Albariño B (51707) and Alvarinho (VIVC nº 15689 and correspond to 52007 of CAN) were confirmed, as expected from the morphologic data and the analyses of the SSR profiles and comparing with the VIVC.

The comparison of our results with the results of Lacombe *et al.* (2013) also show that the Italian cultivar with prime name Formosa on VIVC (Pirovano 245_#1015 in Lacombe *et al.*, 2013), is an homonym of the Portuguese cultivar Formosa.

The cultivar Limnio is often confused with the cultivar Limniona, which genetic profile was included in the Greek Vitis database by Lefort and Roubelakis-Angelakis, (2001). The Vitis International Variety Catalogue (VIVC) includes two Limniona cultivars as homonyms. The accession used in this study has the same SSR profile of the cultivar Limnio (prime name, nº 6835) entered in the VIVC database. This accession is now well identified and can be marketed under the name Limnio by the plant nursery who supplied this material. The genetic profile of cultivar Limnio is clearly different from the Portuguese cultivars analyzed in this study.

The phenetic analysis (Figure 1) show that all the Portuguese cultivars, apart from Barco do Porto (50402), are clustered into two sub groups that differentiate from *Vitis aestivalis* (50417) and the Greek cultivar Limnio. Although there is only one Greek cultivar in this study, this clustering seems to discriminate between "proles occidentalis", that should contain the Portuguese cultivars and "proles pontica", which should include the cultivar Limnio, in agreement with the division by ecotypes proposed by Negrul (1938). Barco de Portugal (50402) is thought to be an inter-specific hybrid.

Identifying synonyms in minority cultivars increases the possibility of obtaining adequate plant material for certification purposes and commercialization. For that purpose, the 19 SSR loci profiles will be added to the Portuguese genotyping database (Veloso *et al.*, 2010) in order to optimize the management of the CAN and to clarify possible existing confusions. This information will be also disclosed to the Vitis International Variety Catalogue (http://www.vivc.de/).

CONCLUSIONS

Genotyping of 28 grapevine accessions using 19 SSR loci revealed eight cases of synonymy, one case of homonymy and one case of mislabeling. Eight of these cultivars (Alvarelhão Ceitão, Boal Espinho, Cornichel Branco, Malvasia Branca de S. Jorge, Molar, Transâncora, Verdial from Vinhos Verdes and Verdial from Douro) are minor cultivars, with no certified plant material. The correct identity and the

detection of synonyms are fundamental for future plant material certification and commercialization, as well as to the knowledge of the diversity of the accessions to be managed and maintained. The intense search of synonyms in minor cultivars increases the possibility of finding plant material in conditions to be candidate for certification control.

Further analysis of the genetic identity of grapevine cultivars is needed in order to optimize the management of the Portuguese National Ampelographic Collection. The obtained information will be proposed to be added to the Portuguese legislation of the grapevine cultivars suited for wine production.

ACKNOWLEDGMENTS

Antonios Alifragkis would like to thank the Alexander S. Onassis Public Benefit Foundation which supported him with a scholarship for the completion of the M.Sc studies. Jorge Cunha is funded by "Fundação para a Ciência e Tecnologia" (SFRH/BPD/74895/2010). This study was carried out in the framework of the European research projects: i) GrapeGen06 "Management & Conservation of Grapevine Genetic Resources" and ii) COST Action FA1003 "East–West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding". Margarida Teixeira-Santos is thanked for helping with the English version of the manuscript.

REFERENCES

Calo A., Costacurta A., Cancellier S., Forti R., 1990. Garnacha, Grenache, Cannonao, Red Tokai, a unique grapevine. *Vingevini*, **17**, 45-48.

Eiras-Dias J.E., Faustino R., Clímaco P., Fernandes P., Cruz A., Cunha J., Veloso M., Castro R., 2011. *Catálogo das castas para vinho cultivadas em Portugal – Volume I.* Instituto da Vinha e do Vinho I.P., Chaves Ferreira – Publicações SA.

Grando M.S., Frisinghelli C., Stefanini M., 2000. Genotyping of local grapevine germplasm. *Acta Hort.*, **528**, 183-187.

Ibáñez J., De Andrés M.T., Molino A., Borrego J., 2003. Genetic study of key spanish grapevine varieties using microsatellite analysis. *Am. J. Enol. Vitic.*, **54**, 22-30.

Ibañez J., Muñoz-Organero G., Zinelabidine L.H., De Andrés M.T., Cabello F., Martíñez-Zapater J.M., 2012. Genetic origin of the grapevine cultivar Tempranillo. *Am. J. Enol. Vitic.*, **63**, 549-553.

Lacombe T., Boursiquot J.-M., Laucou V., Vecchi-Staraz M., Péros J.-P., This P., 2013. Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vini*fera L.). *Theor. Appl. Gene.,* 1-14.

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

Lefort F., Roubelakis-Angelakis K., 2001. Genetic Comparison of Greek Cultivars of *Vitis vinifera* L. by Nuclear Microsatellite Profiling. *Am. J. Enol. Vitic.*, **52**, 101-108.

Lodhi M.A., Ye G.-N., Weeden N.F., Reisch B.I., 1994. A simple and efficient method for DNA extraction from grapevine cultivars and Vitis species. *Plant. Mol. Biol. Rep.*, **12**, 6-13.

Lopes M.S., Santos M.R., Eiras-Dias J.E., Mendonça D., Câmara Machado A., 2006. Discrimination of Portuguese grapevines based on microsatellite markers. *J. Biotechnol.*, **127**, 34-44.

Lopes M.S., Sefc K.M., Eiras-Dias E., Steinkellner H., Laimer Câmara Machado M., Câmara Machado A., 1999. The use of microsatellites for germplasm management in a Portuguese grapevine collection. *Theor. Appl. Gene.*, **99**, 733-739.

Menezes P., 1900. *Apontamentos para o estudo da Ampelographia Portugueza. Boletim da Direcção Geral de Agricultura.* Ministério das Obras Publicas. Comercio e Industria, Imprensa Nacional, Lisboa.

Negrul A.M., 1938. Evolution of cultivated forms of grapes. *CR Acad. Sci. USSR*, **18**, 585-588.

OIV, 2009. *Descriptor list for grapevine varieties and Vitis species*. Organisation Internationale de la Vigne et du Vin (OIV). 2nd edition. Paris, France.

Park S.D.E., 2001. *Trypanotolerance in West african cattle and the population genetic effects of selection*. University of Dublin, Dublin.

Peakall R., Smouse P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, **28**, 2537-2539.

Portaria nº 380/2012, de 22 de Novembro, do Ministério da Agricultura, do Mar, do Ambiente e do Ordenamento do Território. Diário da República, 1.ª série - N.º 226. Lisboa, Portugal.

Santiago J.L., Boso S., Gago P., Alonso-Villaverde V., Martínez M.C., 2007. Molecular and ampelographic characterisation of *Vitis vinifera* L. "Albariño", "Savagnin Blanc" and "Caíño Blanco" shows that they are different cultivars. *Span. J. Agric. Res.*, **5**, 333-340.

Stavrakas E.D., 2011. *Ampelography*. 624 p. Ziti publications, Thessaloniki.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., **28**, 2731-2739.

Veloso M.M., Almandanim M.C., Baleiras-Couto M., Pereira H.S., Carneiro L.C., Fevereiro P., Eiras-Dias J., 2010. Microsatellite database of grapevine (*Vitis vinifera* L.) cultivars used for wine production in Portugal. *Ciência Tec. Vit.*, **25**, 53-61.

Viala P., Vermorel V.,. 1901-10. *Traite generale de viticulture. Ampelographie,* 7 vols. 3146 p. Masson et Cie, Paris.

Supplemental Table I

Characterization of the 28 accessions with 17 OIV morphologic descriptors. Limnio accession was recently introduced on PRT051 collection.

Tabela Suplementar 1. Caracterização dos 28 acessos com 17 descritores morfológicos OIV. O acesso Limnio foi recentemente introduzido na coleção PRT051.

OIV	Description	50402	50417	53805	51502	51401	40405	53210	51707	53209	50108	40604	41809	51202	51805	52513	50614	41206	53807	52115	52017	41201	52206	40303	52112	41207	41208	41209
1*	Young Shoot: Aperture of tip	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4*	Young shoot: density of prostrate hairs on tip	5/7	5	5	5	5/7	1	1	5/7	5	3	3	7	5	5	7	7	7	7	7	7	7	3	3	3	5/7	5	5
16*	Shoot: number of consecutive tendrils	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
51*	Young Leaf: colour of the upper side of the blade (4th leaf)	2	4	1	1	1	3	3	2	2/3	2	2	2/3	2	2/3	1	1	3/4	3	2	2	3/4	3	3	3	2/3	2	3
67*	Mature Leaf: shape of the blade	2	1/2	3	3	3	3	3	4	4	2	4	3	3	3	3/4	3/4	3	3	3	3	3	3	4	4	3	3	2
68*	Mature Leaf:Number of lobes	1	1	3	3	3	3	3	1	3	4	4	3	3	2/3	3	3	3	3	2	2	3	3	3	3	3/4	3	4
70*	Mature leaf: area of anthocyanin colouration of main veins on upper side of blade	1	3	1	1	1	1	1	1/2	3	1	1	1	2	2	1	1	2/3	3/4	1	1	2/3	3	1	1	1	1	1
72	Mature Leaf: Goffering of blade	1	1	1	1	1	1	1	1	1	1	1	1/3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
75	Mature Leaf: Blistering of the upper side of the blade	5	5/7	5	5	5	3	3	5/7	5/7	3/5	3/5	1	3	3	7	7	3/5	3	5	5	5	5	5	5	5	5	5
76*	Mature Leaf:Shape of the teeth	3/5	5	2	2	2	5	5	3	5	2	2	3	3	3	3	3	3	3	3	3	2/3	2	5	5	3	3	3
79*	Mature Leaf: degree of opening / overlaping of petiole sinus	3	3	5	5	5	3	3	3	3	3	3	5/7	5	5	3/5	3/5	3/5	3	5/7	5/7	3/5	3/5	3	3	5	5	5
80	Mature Leaf: Shape of the base of petiole sinus	3	3	2	2	2	3	3	2/3	2/3	1	1	3	3	3	2	2	2/3	3	2/3	2/3	2/3	2	2	2	3	1	3
081 - 2 *	Mature leaf: petiole sinus base limited by veins	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1
84*	Mature leaf: density of prostrate hairs between the main veins on lower side of blade	3	3/5	3	3	3	1	1	3/5	3/5	3	3	3	5	5	5	5	3/5	5	5	5	3/5	5	3	3	1/3	3	3
87*	Mature leaf: density of erect hairs on main veins on lower side of blade	1	1	5	5	5	1	1	1/3	1/3	1	1	3	3	3	1	1	3	1	1	1	3	3	7	7	1/3	7	3/5
223*	Berry: shape	2	1/2	3	3	3	10	10	3	2	2	2	2	3	3	2	2	2	2	1	1	2	2	3	3	2/3	3	2
225*	Berry: colour of skin			6	6	6	1	1	1	1	1	1	6	6	6	1	1	6	6	1	1	6	6	1	1	6	5/6	6

* Priority primary descriptors list