

MICROSATELLITE DATABASE OF GRAPEVINE (*Vitis vinifera L.*) CULTIVARS USED FOR WINE PRODUCTION IN PORTUGAL

BASE DE DADOS DE MICROSATÉLITES DAS CASTAS (*Vitis vinifera L.*) UTILIZADAS NA PRODUÇÃO DE VINHO EM PORTUGAL

Maria Manuela Veloso^{1*}, Maria Cecília Almandanim², Margarida Baleiras-Couto³, Helena Sofia Pereira⁴, Luís Cruz Carneiro¹, Pedro Fevereiro², José Eiras-Dias³

1) INRB – INIA, Oeiras, Quinta do Marquês, 2784 – 505 Oeiras (Portugal)

2) ITQB, Universidade Nova de Lisboa, Av. Da República, Apt.127, 2781 – 901 Oeiras, Portugal

3) INRB – INIA, Dois Portos, Quinta da Almoinha, 2565 -191 Dois Portos, Portugal

4) Centro de Botânica Aplicada à Agricultura, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

* Corresponding author: +351 21 4403500, email: mveloso.inrb@gmail.com

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SUMMARY

Six nuclear microsatellite loci (VVMD 5, VVMD 7, VVMD 27, VrZAG 62, VrZAG 79 and VVS 2) were chosen to study the differentiation of 313 grapevine cultivars officially authorized for wine production in Portugal (Portaria nº 428/2000, Diário da República nº 163, 17th July 2000). These loci were found to be highly informative and therefore constitute a useful marker set for the discrimination of the targeted cultivars. This study allowed for the detection of 244 distinct genotypes as well as for the identification of synonyms in 40 cultivars, where 2 to 6 (synonymous) cultivars represent seventeen genotypes. The remaining 29 cultivars are distributed in 11 distinct sets, where each set displays identical SSRs profiles. However, these should be considered as distinct cultivars considering that these differ in berry colour. Taken together, the results of this work will contribute to improve the management of the Portuguese Grapevine National Collection as well as give a good technical support for the update of the list of cultivars officially authorized for wine production in Portugal (Portaria nº 428/2000). Furthermore, it will also be a useful for the control of plant material in the future.

The SSR database will be available at the web page of Instituto Nacional de Recursos Biológicos (INRB) www.inrb.pt

RESUMO

Trezentas e treze castas referidas na Portaria nº 428/2000 foram genotipadas usando seis loci de microsatélites nucleares (VVMD 5, VVMD 7, VVMD 27, VrZAG 62, VrZAG 79 e VVS 2). Concluiu-se que estes loci eram muito informativos, tendo permitido discriminar as cultivares estudadas. Assim, este estudo possibilitou a identificação de 244 genótipos distintos e 17 outros genótipos, cada um representado, por 2 a 6 castas (sinónimos), num total de 40 cultivares. Há ainda 29 cultivares, distribuídas por 11 grupos distintos, que embora tenham perfis de microsatélites idênticos têm a cor do bago distinta pelo que, não foram incluídas no grupo dos sinónimos.

Este trabalho contribuirá para melhorar a gestão da Coleção Ampelográfica Nacional e constituirá uma boa base de apoio técnico à alteração da lista oficial das castas usadas na produção de vinho em Portugal. É também de salientar a importância que estes resultados têm para, no futuro, apoiar o controlo da comercialização do material vegetal.

A base de dados dos microsatélites estará disponível na página web do Instituto Nacional de Recursos Biológicos (INRB) www.inrb.pt

Key words: autochthonous grapevine, cultivar synonymous, Grapevine Portuguese Collection

Palavras-Chave: videiras autóctones, sinonímias, Coleção Ampelográfica Nacional

INTRODUCTION

Grapevine (*Vitis vinifera L.*) is composed of a large number of cultivars and is one of the oldest crops in the world. Grapes have been cultivated in Portugal for a long time, with evidence that *Vitis* expansion in the region now known as Portugal occurred some 5000 years ago and that the Romans played an important role in the Lusitanian viticulture, namely by the introduction of new cultivars.

Portugal still has a great diversity of autochthonous grapevine cultivars, some of them probably originating from the local wild germplasm (Cunha *et al.*, 2010). Although approximately three hundred

cultivars are officially recognized, nowadays many of them are hardly used and at risk of extinction (Almandanim *et al.*, 2007). Indeed, less than 15 native cultivars represent the majority of those presently utilised for viticulture, namely Alvarinho, Antão Vaz, Arinto, Fernão Pires, for the green yellow cultivars (25 800 ha) and Baga, Castelão, Tinta Barroca, Tinto Cão, Touriga Franca, Touriga Nacional and Trinca-deira, for the blue black cultivars (73 630 ha). Others, not of Portuguese origin, like Aragonez (23 500 ha) are also of great importance.

The preservation of important genetic diversity represented by the Portuguese cultivars started in 1988

with the establishment of the Portuguese Grapevine National Collection containing approximately 720 cultivars, including all the autochthonous ones. This Collection is of great significance and imperative for ampelographic studies involving the resolution of synonyms and homonyms problems, both nationally and internationally.

The high number of cultivars utilized in Portugal and their dissemination all over the country resulted in different names being attributed to genetically identical plants (synonymous), which is a problem for viticulture and for the germplasm management.

Traditionally, cultivar characterization relied on plant morphological description (Eiras-Dias *et al.*, 1988). However, these observations are time consuming and error-prone due to environmental variations that may alter the expression of the measured characteristics.

In the last years, developments in DNA analysis for the discrimination of cultivars through the application of the microsatellite (SSR) fingerprinting in viticulture has become the technique of choice for cultivar identification and distinction (Bowers *et al.*, 1996; Sefc *et al.*, 1999). According to the OIV, SSR are the best markers to discriminate the cultivars. In fact, This *et al.*, 2004 demonstrated the usefulness of a standard set of microsatellite for identification of grape cultivars

The present study intended to complement detailed ampelographic characterization of grapevine germplasm resources by using six microsatellite loci to discriminate 313 grapevine accessions. The results allowed the identification of duplications and/or redundancies in the Collection as well as the completion of a public database.

MATERIALS AND METHODS

Plant material

Three hundred and thirteen accessions of *V. vinifera* ssp. *vinifera* L. from the Portuguese Grapevine National Collection (PRT 051) located at Dois Portos (Quinta da Almoína) were analysed in this study. All these cultivars are officially authorized for wine production in Portugal (Portaria nº 428/2000, Diário da República nº 163, 17th July 2000). Although the Portaria lists 341 cultivars, 28 were not analysed because they did not integrate in the National Collection. Although most of the cultivars studied are autochthonous, some are of foreign origin.

Table I, Table II and Table III list all the accessions with the indication of their Reference in the National Collection and in the official journal, and the colour of the berry according to the OIV descriptor list for grape varieties and the *Vitis* species (blue black - N, green yellow - B, rose - Rs).

Microsatellite analysis

DNA extraction, PCR amplification, electrophoresis and detection of polymorphisms were carried out according to Almandanim *et al.*, (2007). Six microsatellite loci recommended by the OIV, considered the minimal standard marker set for grapevine cultivar analysis (This *et al.*, 2004), VVMD 5 and VVMD 7 (Bowers *et al.*, 1996), VVMD 27 (Bowers *et al.*, 1999), VrZAG 62 and VrZAG 79 (Sefc *et al.*, 1999) and VVS 2 (Thomas and Scott, 1993), were successfully amplified. Allelic size determination was carried out through capillary electrophoresis in the CEQ8000 Genetic Analysis System (Beckman Coulter).

RESULTS AND DISCUSSION

Three hundred and thirteen grapevine cultivars officially authorized for wine production (Tables I, II and III) were genotyped at 6 SSRs loci. Their berry colour is blue black (50%), green yellow (44%) or rose (6%). The genotyping yielded 66 alleles, ranging from 8 (VVMD 27 and VrZAG 62) to 15 (VVS2) alleles per locus. The genetic diversity found is consistent with previous studies (Lopes *et al.*, 2006, Almandanim *et al.*, 2007, Cunha *et al.*, 2010).

Table I lists 244 cultivars which, by the SSR profiles, correspond to distinct genotypes. The remaining 69 cultivars include 40 which are officially registered under different names, but that, correspond only to 17 distinct genotypes due to the presence of synonymous (Table II). In this group a single cultivar is registered under two to six distinct names as, for example, Ramisco-Tinto, Rabo-de-Ovelha-Tinto, Saborinho, Molar, Tinto-de-Porto-Santo e Tinta-Negra, which unambiguously refer to one single genotype. Síria and Sabro were also considered as synonymous, although they have one allele (VVS2) with a reproducible difference on two bases. As they have the same ampelographic characteristics, we considered this difference as a mutation. The other 29 cultivars can be distributed in 11 distinct sets, the cultivars of each set having identical SSRs profile but different berry colour (Table III). For instance, Fernão-Pires and Fernão-Pires-Rosado have the same SSR profiles but Fernão-Pires have green yellow berries while Fernão-Pires-Rosado has rose berries.

In fact, the berry colour types are indistinguishable by microsatellite analysis as already reported for some of the Portuguese cultivars (Lopes *et al.*, 1999). According to the Portuguese official legislation, cultivars with different berry colour should be considered as distinct cultivars.

Concerning the origin of the distinct cultivars described in the present work, we could arrange them in four groups: 1) autochthonous to Portugal (almost 50%); 2) hybrids obtained at Estação Agronómica Nacional by the researcher Leão Ferreira de Almeida, corresponding to 29 cultivars; 3) common to other

TABLE I

Genetic profiles, at 6 microsatellite loci, of 244 grapevines officially authorized for wine production in Portugal, for which no synonymous were detected. The cultivars are referred by the official name, the reference number at Coleção Ampelográfica Nacional and the Portaria number. Allele size is given in base pairs. B, N and Rs correspond to green yellow, blue black and rose colour of the berry, respectively
Tamanho dos alelos para seis loci de microssatélites de 244 castas oficialmente autorizadas para a produção de vinho em Portugal. As castas são referidas pelo nome oficial, referência na Coleção Ampelográfica Nacional e na Portaria nº 428/2000. B, N e Rs correspondem a castas brancas, tintas e rosadas, respectivamente

Cultivar Name	Collection Reference	Portaria Number	VVMD 5		VVMD 7		VVMD 27		VrZAG 62		VrZAG 79		VVS 2	
			allele 1	allele 2										
Agronómica N	41505	1	236	238	239	243	179	185	188	192	239	251	137	145
Aqua Santa N	50615	2	238	238	239	249	179	189	188	200	251	251	147	147
Alcoa N	41504	3	226	238	235	253	181	181	188	188	243	247	141	145
Alicante-Bouschet N	53808	5	226	238	235	239	181	194	188	188	243	257	135	147
Alicante-Branco B	50711	6	228	240	235	239	179	194	186	188	251	257	145	147
Almafra B	52313	7	222	226	235	245	179	181	188	204	247	251	139	147
Alvadurão B	52114	9	222	236	235	245	181	181	194	204	247	247	139	153
Alvarelhão N	53207	12	222	226	235	235	185	189	188	194	251	259	135	153
Alvarelhao-Ceitão N	41209	14	226	240	235	239	189	194	188	188	251	257	145	153
Alvarinhão B	52007	15	222	232	235	235	189	189	186	204	247	251	137	153
Amor-Nao-Me-Deixes N	51003	17	234	234	235	239	181	194	188	196	243	257	135	145
Amostrinha N	53204	18	234	238	239	253	181	189	188	188	247	251	145	147
Antão-Vaz B	52316	19	234	236	245	259	181	183	204	204	247	247	147	153
Aragonez N	52603	20	236	236	235	249	183	183	196	200	247	251	145	147
Aramon N	53704	21	226	226	243	245	179	181	204	204	251	259	135	135
Arinto B	52311	22	226	238	239	247	181	185	186	188	247	251	145	153
Arinto-do-Interior B	51412	23	222	240	235	235	183	194	186	188	247	247	145	153
Arjunção N	52104	25	228	240	235	245	185	189	194	196	243	251	153	159
Arnsburguer B	40602	26	226	236	243	243	181	189	194	204	243	259	135	145
Assaraky B	40404	27	222	226	235	235	183	185	186	188	243	247	135	137
Avesso B	52310	28	222	240	235	235	181	189	186	186	243	247	137	153
Azal B	52809	29	226	232	235	243	181	185	194	204	247	251	153	159
Babosa B	40603	30	226	240	243	249	179	181	188	202	243	247	145	147
Baga N	52606	31	232	240	235	235	179	189	188	204	247	251	145	157
Barca N	52101	32	226	232	235	235	183	189	188	188	245	257	145	153
Barcelo B	52407	33	226	232	235	259	181	189	194	196	245	247	137	153
Bastardo-Branco B	51117	36	226	238	253	253	175	189	188	188	247	251	145	153
Batoca B	52507	39	226	226	243	247	183	185	186	204	247	251	145	153
Beba B	51808	40	236	240	239	245	179	189	188	204	243	247	137	145
Bical B	52016	41	226	240	235	259	179	185	188	194	251	251	135	147
Boal-Branco B	52116	43	238	240	249	253	179	185	188	188	251	251	141	145
Boal-Espinho B	52017	44	226	234	235	239	179	194	188	188	253	257	145	147
Bonvedro N	41601	45	226	240	239	249	181	181	194	200	247	251	145	147
Borraçal N	52807	46	232	238	235	235	181	185	194	194	247	247	135	137
Branca-de-Anadia B	50314	48	226	238	235	249	183	185	188	188	247	257	147	153
Branco-Desconhecido B	41107	49	226	240	235	245	181	194	196	204	249	259	135	137
Branco-Especial B	51216	50	228	236	239	243	181	189	188	194	245	259	153	157
Branco-Guimarães B	51018	52	226	232	235	235	185	185	186	186	243	251	135	159
Branda B	52117	54	226	226	235	253	183	189	188	194	247	251	135	145
Brando N	41202	55	226	226	259	259	183	189	188	194	245	247	145	159
Cabernet-Franc N	50801	57	226	240	237	261	181	189	194	204	247	259	141	149
Cabinda N	53103	58	226	236	235	253	179	181	188	188	243	251	147	153
Cabernet-Sauvignon N	53606	58	232	240	235	235	181	189	188	194	247	247	141	153
Caladoc N	50102	61	224	236	235	235	191	194	188	188	257	259	147	153
Camarate N	52402	63	234	236	239	249	181	189	188	200	247	251	147	153
Campanário N	41806	64	226	238	235	253	181	183	188	194	245	247	147	153
Caracol B	50914	65	240	240	235	243	179	194	188	196	247	257	135	147
Caramela B	51016	66	228	236	243	243	185	185	192	194	239	251	137	147
Carginan N	53804	68	226	228	235	235	181	185	186	188	251	259	145	147
Carrasqueno B	52605	69	222	226	235	253	179	189	188	188	251	261	135	147
Carrega-Branco B	51816	70	234	236	239	253	181	189	188	194	245	247	139	153
Carrega-Burros N	52902	71	228	234	235	239	181	185	188	188	247	251	139	145
Cascal B	51517	73	222	226	235	243	181	181	186	204	247	247	153	153
Cascaulho N	50901	74	236	238	245	249	181	189	200	204	247	251	147	153
Castalia B	40702	75	228	238	245	249	185	194	188	196	247	257	145	151
Castelã N	51002	76	236	238	245	253	181	181	188	204	247	251	139	153
Castelão N	53106	77	236	238	239	253	179	181	188	188	247	251	145	147
Castelão-Branco B	52615	78	226	240	235	253	181	189	188	188	247	251	145	153
Castelino N	52706	79	232	232	249	253	179	194	194	200	243	257	135	135
Castelo-Branco B	50309	80	238	240	235	249	181	183	188	194	247	247	145	147
Casteloa N	41303	81	234	238	245	249	181	189	200	204	247	251	139	145
Cerceal-Branco B	52410	82	226	236	245	253	179	181	188	204	247	251	145	159
Cercial B	52412	83	226	240	251	255	179	185	188	188	251	259	147	153

Cultivar Name	Collection Reference	Portaria Number	VVMD 5		VVMD 7		VVMD 27		VrZAG 62		VrZAG 79		VVS 2	
			allele 1	allele 2										
Chardonnay B	53511	84	234	238	235	239	181	189	188	196	243	245	139	145
Chemin B	53512	89	228	232	235	253	175	189	188	194	247	251	135	153
Cidelhel N	51308	90	232	234	239	249	181	194	192	200	247	257	145	145
Cidreiro N	51404	91	226	232	235	249	181	181	188	200	247	259	153	153
Côdega-de-Larinho B	51317	93	222	240	235	239	189	194	186	188	243	257	135	147
Colombard B	50114	94	232	240	235	235	175	181	182	184	243	247	145	153
Complexa N	50201	95	226	238	235	245	185	194	186	188	239	257	147	151
Concieira N	50902	96	238	238	235	249	181	181	188	200	247	251	135	153
Coração-de-Galo N	51304	97	226	232	235	235	181	189	188	194	245	247	135	145
Cornichon B	40708	98	236	246	243	245	179	181	196	204	251	251	147	151
Cornifesto N	52004	99	234	238	245	249	181	189	200	204	247	251	145	147
Corropio N	51405	100	228	238	235	249	185	194	186	188	247	257	135	135
Corvo N	51207	102	232	236	249	253	179	194	194	200	251	251	129	135
Dedo-de-Dama B	51209	104	236	236	243	245	179	181	196	204	251	251	147	151
Deliciosa N	41707	105	226	238	235	249	181	185	188	188	243	257	145	147
Diagalves B	52513	106	236	240	239	249	181	185	188	188	247	247	145	153
Doçal N	50904	107	226	238	243	259	183	194	186	194	247	251	145	153
Doce N	50905	108	226	226	247	259	183	189	186	194	247	247	135	145
Dona-Joaquina B	51609	110	226	232	235	245	179	194	186	188	247	251	135	153
Donzelinho-Branco B	52307	111	232	238	239	259	189	189	188	194	251	251	141	153
Donzelinho-Roxo Rs	41709	112	228	238	235	239	189	194	186	192	251	257	145	153
Donzelinho-Tinto N	52306	113	226	232	239	259	181	189	194	194	251	251	153	153
Dorinto B	51411	114	238	240	235	253	181	189	188	194	247	251	137	153
Encruzado B	52207	115	226	232	235	253	183	189	194	194	247	251	151	153
Engomada N	51008	116	232	238	235	235	181	194	188	188	247	257	145	145
Esganinho B	41103	118	232	238	235	259	181	189	188	194	245	247	141	153
Esganoso N	50915	119	226	236	235	235	181	181	188	194	239	247	135	153
Espadairo N	52904	120	222	226	243	259	183	189	196	204	251	251	135	153
Espadairo-Mole N	51604	121	236	240	235	245	189	189	194	200	251	251	135	157
Estreito-Macio B	51017	122	222	236	235	239	181	189	188	196	247	251	135	147
Fepiro N	41502	124	226	226	235	239	181	194	188	188	243	247	141	153
Ferral N	50104	127	232	238	235	245	183	194	192	204	247	257	137	149
Fontecal B	52314	131	226	234	235	235	183	185	186	186	247	251	135	153
Galego N	41203	132	228	232	239	251	185	189	200	204	239	251	133	141
Galego-Dourado B	52913	133	228	240	235	239	185	189	188	194	245	251	135	135
Gamay N	53906	135	234	238	235	245	181	189	194	204	243	245	135	139
Generosa B	40808	136	226	228	235	235	194	194	186	194	247	247	147	153
Gewurztraminer Rs	53904	137	232	238	239	253	189	189	188	194	245	251	153	153
Goncalo-Pires N	50802	140	226	236	235	239	181	181	188	196	247	259	135	145
Gorda N	50607	141	234	238	245	253	181	189	188	204	247	251	139	153
Gouveio-Estimado B	50617	143	226	234	239	245	181	189	188	204	247	251	139	153
Gouveio-Preto N	41305	144	236	238	235	235	183	189	186	196	245	251	145	153
Gouveio-Real B	50616	145	226	234	235	239	181	189	188	204	247	251	145	153
Grand-Noir N	50804	148	226	234	235	239	181	183	188	188	243	259	141	153
Grangeal N	51602	149	226	236	235	235	181	189	186	194	247	261	153	153
Granho B	40606	150	236	240	235	239	181	194	188	194	247	247	139	147
Grenache N	53406	151	226	240	235	239	194	194	188	188	257	257	139	147
Jacquere B	40806	153	226	234	235	245	179	181	196	204	243	251	135	145
Jaen N	52503	154	226	236	245	253	181	189	188	194	247	251	147	153
Labrusco N	41204	156	226	238	245	259	181	185	194	200	243	247	153	157
Lameiro B	50611	157	230	232	235	235	181	185	188	194	247	251	135	159
Larião B	51113	158	238	240	235	235	181	194	188	192	257	257	147	149
Lilás B	40701	160	232	238	235	239	185	194	188	196	251	257	153	153
Listrão Rs	41605	161	228	240	235	245	185	189	194	196	243	251	135	137
Loureiro B	52213	161	232	232	247	259	181	185	186	196	251	251	145	153
Lourela N	50708	163	232	240	239	259	181	189	188	194	247	247	141	145
Lusitano N	41503	164	238	238	235	253	181	194	188	188	247	257	145	147
Luzidio B	51115	165	226	232	235	235	181	189	188	204	245	247	145	153
Malandra N	50608	166	226	240	235	249	179	181	188	200	261	261	145	153
Malvarisco N	53308	167	226	232	249	259	185	189	188	200	251	251	135	153
Malvasia B	52714	168	232	234	235	235	181	181	188	194	247	257	145	147
Malvasia-Bianca B	51009	169	226	238	245	259	185	185	200	202	239	251	135	145
Malvasia-Branca B	50912	170	226	238	239	245	179	179	188	200	255	257	135	139
Malvasia-Branca-de-São-Jorge B	40604	171	222	226	235	235	181	194	188	196	247	251	135	147
Malvasia-Cabral Rs	51212	172	222	240	235	245	183	189	188	204	247	251	139	153
Malvasia-Rei B	53013	179	228	240	235	245	185	194	188	194	251	257	135	147
Malvoeira B	40704	182	226	238	235	239	185	194	188	204	247	257	145	147
Manteúdo B	51413	183	222	226	235	245	181	181	188	194	237	259	145	145
Mateúdo-Preto N	41603	184	228	234	249	249	181	189	194	204	243	247	133	145
Marquinhas B	53312	186	226	228	235	235	194	194	188	188	247	247	153	153
Melra N	41309	189	228	236	235	235	181	194	188	188	245	257	147	153
Merlot N	50518	190	226	236	235	243	189	191	194	194	259	259	141	153
Mindelo N	41607	191	238	238	235	245	179	185	186	188	247	247	137	147

Cultivar Name	Collection Reference	Portaria Number	VVMD 5		VVMD 7		VVMD 27		VrZAG 62		VrZAG 79		VVS 2	
			allele 1	allele 2										
Mondet N	50702	194	232	232	235	247	179	191	194	200	251	251	135	153
Monvedro N	51804	195	226	226	235	249	179	189	188	200	245	251	153	153
Moreto N	52301	196	226	236	239	253	181	189	188	188	247	251	147	153
Moscadet B	51417	197	232	234	235	235	175	179	188	196	237	247	135	135
Moscargo N	41508	198	238	238	243	253	179	179	188	192	251	255	145	151
Moscatel-Galego-Tinto N	41301	201	226	228	235	245	179	189	186	188	251	255	135	153
Moscatel-Graúdo B	40705	202	228	232	245	247	179	194	186	204	247	255	135	151
Moscatel-Nunes B	53015	203	232	234	235	245	179	194	186	188	255	257	135	147
Mourisco N	51701	204	226	226	259	259	181	189	194	196	247	259	137	157
Mourisco-Branco B	50916	205	234	240	235	239	181	194	188	188	247	257	145	147
Mourisco-de-Semente N	51402	206	232	238	235	235	181	194	188	194	247	257	137	145
Mourisco-de-Trevões N	41306	207	232	238	235	247	194	194	188	200	257	257	135	147
Mulata N	53407	209	226	236	235	253	179	181	188	188	243	251	147	147
Muller-Thurgau B	53313	210	226	228	243	253	181	181	194	194	243	245	145	153
Naia B	40703	211	234	240	235	235	185	194	188	204	251	253	137	147
Negra-Mole N	52202	212	222	240	235	235	181	181	188	196	247	259	145	159
Nevoeira N	52005	213	232	236	235	253	179	189	186	194	247	247	153	159
Padeiro N	50806	214	226	234	235	235	189	194	188	188	251	257	137	147
Parreira-Matias N	52702	215	224	224	249	253	179	181	188	200	251	259	145	153
Patorra N	52006	216	236	240	235	245	181	189	188	194	243	247	135	147
Pé-Comprido B	41002	218	226	226	235	235	185	189	186	196	245	251	135	135
Pedral N	52105	219	226	226	235	259	181	185	186	196	247	251	153	159
Perrum B	51617	222	236	240	235	239	181	185	188	188	243	247	135	147
Petit-Bouschet N	51206	223	234	238	235	239	181	189	188	196	245	247	135	153
Pical N	51007	226	226	232	235	239	179	189	188	188	251	251	135	135
Pilongo N	51606	227	238	240	235	253	179	185	186	194	247	247	135	145
Pintosa B	51217	233	226	232	239	247	179	185	186	188	251	251	145	153
Portugues-Azul N	50605	234	226	232	239	251	181	194	188	204	249	259	145	153
Praça B	51715	235	226	232	235	235	185	189	186	186	251	251	135	159
Preto-Cardana N	52705	236	232	236	245	253	181	181	188	194	245	247	139	153
Preto-Martinho N	51803	237	232	238	235	249	179	183	188	200	247	251	145	147
Primavera N	53102	238	226	236	239	239	181	181	188	188	243	247	147	147
Promissão B	40501	239	234	236	229	239	179	185	188	196	239	249	135	145
Rabigato B	52014	240	222	232	235	235	185	189	186	196	243	251	135	135
Rabigato-Franco B	51613	241	228	236	243	249	185	185	188	194	239	251	135	145
Rabigato-Moreno B	50917	242	232	234	235	235	185	194	186	188	237	247	135	145
Rabo-de-Anho N	52903	243	222	226	235	259	181	185	186	196	247	251	137	159
Rabo-de-Ovelha B	52011	245	222	236	235	239	181	181	188	194	247	247	139	153
Ramisco N	52203	247	226	238	235	259	181	185	188	196	247	251	135	159
Ratinho B	52309	249	222	240	245	253	181	194	188	204	247	247	139	147
Ricoca N	51103	250	232	238	235	239	183	189	188	192	245	247	145	145
Riesling B	53209	251	226	234	245	253	181	189	194	204	243	245	145	153
Rio-Grande B	40809	252	238	240	235	239	181	183	188	188	247	247	145	153
Roal Rs	53806	253	234	236	245	249	181	183	188	204	247	247	139	145
Rodo N	51708	254	226	232	235	257	189	189	194	194	245	259	135	141
Roseira N	50707	255	226	232	235	249	181	194	188	200	247	257	145	145
Roupeiro-Branco B	51314	256	240	240	235	243	185	194	188	204	245	257	145	153
Roxo-Flor Rs	41705	257	226	232	239	239	185	194	188	188	251	257	145	153
Roxo-Rei Rs	50919	258	226	228	235	235	183	185	188	188	247	259	135	145
Rufete N	52106	259	226	236	235	253	181	189	188	194	245	247	135	159
Santareno N	52304	263	228	234	235	239	179	189	188	194	251	255	135	153
São-Mamede B	51611	265	230	236	235	245	185	189	188	200	251	251	135	157
Sarigo B	51316	267	234	236	239	245	181	181	188	204	247	247	139	147
Sauvignon B	53211	268	228	232	235	253	175	189	188	194	245	247	135	153
Seara-Nova B	40403	269	238	240	235	239	181	183	188	194	247	247	145	147
Semilão B	50809	270	232	240	235	235	175	181	188	196	243	247	145	153
Semillon B	53212	271	236	238	235	253	175	185	188	194	247	247	135	135
Sercial B	40505	272	226	238	235	249	181	185	188	194	247	259	135	153
Sercialinho B	51011	273	226	240	235	235	181	194	188	188	247	251	147	153
Sevilhão N	51403	274	228	238	245	259	189	189	194	200	251	259	153	153
Sousão N	51901	276	222	226	235	235	185	189	194	196	245	251	137	153
Syrah N	41407	277	226	232	235	235	189	191	188	194	245	251	135	135
Tália B	52910	278	226	232	245	249	179	183	194	200	245	251	135	145
Tannat N	41609	280	238	240	245	245	185	189	194	200	239	251	145	157
Teinturier N	53807	281	226	236	235	235	181	185	188	196	245	253	147	153
Terrantez B	52210	282	226	238	243	259	185	189	194	196	251	251	145	159
Terrantez-da-Terceira B	50218	283	222	226	235	253	179	189	188	194	251	251	147	153
Terrantez-do-Pico B	50216	284	232	238	235	235	175	189	188	196	247	251	145	153
Tinta-Aguiar N	50703	286	226	232	235	239	189	194	192	194	245	257	145	147
Tinta-Aurélia B	40609	287	232	238	235	253	189	194	188	200	251	257	147	153
Tinta-Barroca N	52905	288	228	236	235	239	181	183	188	192	245	247	145	153
Tinta-Carvalha N	52201	291	232	236	245	259	181	189	194	204	247	251	147	153
Tinta-Fontes N	50706	292	226	238	235	247	181	189	186	188	245	247	153	153

Cultivar Name	Collection Reference	Portaria Number	VVMD 5		VVMD 7		VVMD 27		VrZAG 62		VrZAG 79		VVS 2	
			allele 1	allele 2										
Tinta-Francisca N	52502	293	238	238	235	235	185	189	186	188	243	247	135	135
Tinta-Martins N	50602	296	232	238	235	239	183	194	188	192	257	259	147	155
Tinta-Mesquita N	50604	297	226	232	235	259	189	194	188	196	245	257	135	147
Tinta-Miúda N	51906	298	226	238	235	235	179	183	186	188	251	259	141	153
Tinta-Penajoia N	51208	300	226	232	247	253	179	189	194	200	247	251	135	153
Tinta-Pereira N	50907	301	232	236	235	245	181	185	188	204	247	259	147	159
Tinta-Pomar N	50807	302	238	238	247	253	189	194	200	200	251	257	137	153
Tinta-Tabuaço N	51307	304	236	238	235	253	175	183	186	188	247	251	145	159
Tintem N	52505	305	238	238	235	253	181	181	188	188	247	257	135	145
Tintinha N	51205	306	234	238	239	253	181	181	188	188	243	247	135	147
Tinto-Cão N	53307	307	232	234	235	259	181	185	186	194	247	251	135	135
Tinto-Pegões N	52506	308	236	238	235	239	181	181	188	188	243	247	135	147
Touriga-Fêmea N	50705	311	236	240	235	253	179	189	188	194	245	251	145	145
Touriga-Franca N	52205	312	226	228	235	239	181	183	192	194	245	247	145	153
Touriga-Nacional N	52206	313	226	236	235	235	181	189	188	194	245	245	145	153
Trajadura B	52710	314	226	236	235	247	181	185	186	186	247	247	145	153
Transancora N	41206	315	226	232	235	259	189	189	188	196	245	251	137	159
Trigueira Rs	50909	316	238	240	235	253	181	189	188	188	245	247	147	153
Trincadeira N	53006	317	234	238	235	245	181	185	188	204	247	251	135	153
Trincadeira-Branca B	51012	318	234	238	235	235	175	181	188	188	247	247	145	147
Trincadeira-das-Pratas B	52216	319	238	240	235	253	189	194	188	188	251	257	143	145
Triunfo N	41509	320	236	238	239	243	181	181	188	192	247	255	147	151
Uva-Cão B	51415	321	222	232	235	235	181	189	188	196	247	251	135	153
Uva-Cavaco B	51211	322	234	238	235	235	181	191	186	188	247	251	139	153
Valbom N	53206	324	236	238	235	253	181	181	188	188	243	247	147	147
Valdosa N	51608	325	232	234	235	249	181	181	188	200	247	247	145	147
Varejoa N	50808	328	222	226	235	243	181	181	186	196	247	247	135	153
Vencedor B	52111	329	226	236	235	245	179	181	188	204	247	251	139	147
Verdelho-Tinto N	51806	332	228	236	235	245	181	189	188	200	251	259	135	145
Verdial-Tinto N	41208	334	226	228	235	235	179	183	188	188	247	251	145	147
Vinhão N	51902	335	222	226	235	259	189	189	188	196	245	251	135	137
Viognier B	40807	336	226	232	235	245	185	191	188	200	251	251	135	141
Viosinho B	52715	337	232	232	235	239	185	189	186	188	243	245	135	153
Vital B	52614	338	222	240	235	235	181	194	188	188	247	253	147	153
Zinfandel N	41409	341	226	236	243	245	179	181	200	204	237	259	135	145

TABLE II

Genetic profiles, at 6 microsatellite loci, of 40 grapevine cultivars officially authorized for wine production in Portugal for which 17 synonymous were detected. The cultivars are referred by the name, the reference number at Coleção Ampelográfica Nacional and the Portaria number. Allele size are given in base pairs. B, N and Rs correspond to green yellow, blue black and rose colour of the berry, respectively.
Tamanho dos alelos para seis loci de microssatélites de 40 castas oficialmente autorizadas para a produção de vinho em Portugal e para as quais foram identificadas 17 sinônimas. As castas são referidas pelo nome oficial, referência na Coleção Ampelográfica Nacional e na Portaria nº 428/2000. B, N e Rs correspondem a castas brancas, tintas e rosadas respectivamente.

Cultivar Name	Collection Reference	Portaria Number	VVMD 5		VVMD 7		VVMD 27		VrZAG 62		VrZAG 79		VVS 2	
			allele 1	allele 2										
Alfrocheiro N	52003	4	226	238	249	253	179	189	188	200	251	251	145	153
Tinta-Bastardinha N	51107	289	226	238	249	253	179	189	188	200	251	251	145	153
Alvarelhão-Branco B	51118	13	226	232	235	259	179	189	188	194	251	251	135	153
Branco-Gouvéas B	41105	51	226	232	235	259	179	189	188	194	251	251	135	153
Touriga-Branca B	51515	310	226	232	235	259	179	189	188	194	251	251	135	153
Amaral N	52907	16	222	232	235	259	189	189	188	196	245	251	145	159
Melhorio N	41205	188	222	232	235	259	189	189	188	196	245	251	145	159
Barreto N	41302	34	228	238	239	239	183	189	188	192	251	257	147	159
Bragão N	41307	47	228	238	239	239	183	189	188	192	251	257	147	159
Bastardo-Tinto N	51108	38	222	238	235	245	175	181	188	204	247	247	139	153
Tinta-Lisboa N	51407	295	222	238	235	245	175	181	188	204	247	247	139	153
Carrega-Tinto N	51603	72	234	238	235	249	179	194	188	200	251	257	145	153
Grossa N	52906	152	234	238	235	249	179	194	188	200	251	257	145	153
Cinsaut N	53805	92	226	226	243	247	179	181	188	204	255	259	127	133
São-Saul N	51502	266	226	226	243	247	179	181	188	204	255	259	127	133
Dona-Branca B	51717	109	236	240	235	235	179	185	186	186	251	251	145	159
Folha-de-Figueira B	51514	130	236	240	235	235	179	185	186	186	251	251	145	159
Farinheira B	41304	123	226	232	235	253	181	189	188	194	247	251	135	145
Malvasia-Parda B	50913	177	226	232	235	253	181	189	188	194	247	251	135	145
Gigante B	40504	138	234	240	235	245	181	181	196	204	237	243	135	145
Valente B	40502	326	234	240	235	245	181	181	196	204	237	243	135	145
Marufo N	52002	187	228	232	235	239	183	194	188	192	247	257	145	147
Mourisco-Roxo Rs	52001	208	228	232	235	239	183	194	188	192	247	257	145	147
Molar N	52703	192	226	238	235	253	181	189	188	194	245	261	135	153
Rabo-de-Ovelha-Tinto N	51703	246	226	238	235	253	181	189	188	194	245	261	135	153
Ramisco-Tinto N	51503	248	226	238	235	253	181	189	188	194	245	261	135	153
Saborinho N	51711	260	226	238	235	253	181	189	188	194	245	261	135	153
Tinta-Negra N	51202	299	226	238	235	253	181	189	188	194	245	261	135	153
Tinta-Porto-Santo N	41701	303	226	238	235	253	181	189	188	194	245	261	135	153
Molinha B	51911	193	226	236	235	239	179	181	188	194	247	251	147	153
Tamarez B	51910	279	226	236	235	239	179	181	188	194	247	251	147	153
Pau-Ferro N	52103	217	222	238	235	235	179	189	186	188	251	261	135	135
Tinta-Caiada N	51905	290	222	238	235	235	179	189	186	188	251	261	135	135
Tinta-Lameira N	50704	294	222	238	235	235	179	189	186	188	251	261	135	135
Jampal B	52515	155	226	236	239	253	181	189	188	188	247	251	139	145
Pinheira-Branca B	50714	228	226	236	239	253	181	189	188	188	247	251	139	145
Budelho B	40707	56	232	232	235	253	179	189	186	194	245	251	135	153
Samarrinho B	51516	262	232	232	235	253	179	189	186	194	245	251	135	153
Sabro B	51213	261	222	234	235	245	181	181	186	204	247	247	141	153
Síria B	51914	275	222	234	235	245	181	181	186	204	247	247	139	153

TABLE III

Genetic profiles, at 6 microsatellite loci, of 29 grapevine cultivars officially authorized for wine production in Portugal of which 11 have identical SSR profiles but different berry colour. The cultivars are referred by the name, the reference number at Coleção Ampelográfica Nacional and the Portaria number. Allele size are given in base pairs. B, N and Rs correspond to green yellow, blue black and rose colour of the berry, respectively

Tamanho dos alelos para seis loci de microssatélites de 29 castas oficialmente autorizadas para a produção de vinho em Portugal. Nestas castas há 11 que embora tendo o mesmo perfil de SSR têm mutações na cor do bago. As castas são referidas pelo nome oficial, referência na Coleção Ampelográfica Nacional e na Portaria nº 428/2000. B, N e Rs correspondem a castas brancas, tintas e rosadas respectivamente

Cultivar Name	Collection Number	Portaria Number	VVMD 5		VVMD 7		VVMD 27		VrZAG 62		VrZAG 79		VVS 2	
			allele 1	allele 2										
Bastardo N	52803	35	238	238	235	253	175	189	188	188	245	247	145	153
Graciosa N	50304	147	238	238	235	253	175	189	188	188	245	247	145	153
Bastardo-Roxo Rs	41708	37	238	238	235	253	175	189	188	188	245	247	145	153
Carão-de-Moça B	51612	67	232	240	239	243	185	189	194	204	245	251	135	153
Folgasão B	52709	128	232	240	239	243	185	189	194	204	245	251	135	153
Folgasão-Roxo Rs	52708	129	232	240	239	243	185	189	194	204	245	251	135	153
Fernão-Pires B	52810	125	226	240	235	235	183	194	188	194	247	247	147	153
Fernão-Pires-Rosado Rs	52815	126	226	240	235	235	183	194	188	194	247	247	147	153
Godelho B	41101	139	226	238	235	239	185	189	186	188	251	251	153	159
Gouveio B	52112	142	226	238	235	239	185	189	186	188	251	251	153	159
Gouveio-Roxo Rs	41706	146	226	238	235	239	185	189	186	188	251	251	153	159
Malvasia-Fina B	52512	175	226	240	235	253	179	194	188	188	247	251	145	147
Malvasia-Fina-Roxa Rs	52612	176	226	240	235	253	179	194	188	188	247	251	145	147
Malvasia-Cândida B	50911	173	226	226	239	245	179	183	188	202	243	247	145	147
Malvasia-Cândida-Roxa Rs	50810	174	226	226	239	245	179	183	188	202	243	247	145	147
Mário-Feld N	41606	185	228	238	235	239	185	189	188	194	239	245	139	153
Pinot-Blanc B	51713	230	228	238	235	239	185	189	188	194	239	245	139	153
Pinot-Gris Rs	53708	231	228	238	235	239	185	189	188	194	239	245	139	153
Pinot-Noir N	53706	232	228	238	235	239	185	189	188	194	239	245	139	153
Chasselas B	53609	85	228	236	235	243	185	189	194	204	251	259	135	145
Chasselas-Roxo Rs	53607	86	228	236	235	243	185	189	194	204	251	259	135	145
Chasselas-Salsa B	40608	88	228	236	235	243	185	189	194	204	251	259	135	145
Uva-Salsa B	50311	323	228	236	235	243	185	189	194	204	251	259	135	145
Moscatel-Galego-Branco B	52915	199	228	236	229	245	179	194	186	196	251	255	135	135
Moscatel-Galego-Roxo Rs	54005	200	228	236	229	245	179	194	186	196	251	255	135	135
Verdelho B	50317	330	222	232	235	253	181	189	194	196	247	251	135	153
Verdelho-Roxo Rs	51513	331	222	232	235	253	181	189	194	196	247	251	135	153
Malvasia-Preta N	53205	178	226	236	245	249	181	189	200	204	247	251	139	153
Pinheira-Roxa Rs	41703	229	226	236	245	249	181	189	200	204	247	251	139	153

countries, for example Alicante-Branco, Aragonez, Moscatel-Galego-Branco, Moscatel-Graúdo, Tália and Tinta-Miúda; 4) a group including 37 varieties that have recently been introduced (mainly from France and Germany) in the national viticulture, as for example, Cabernet-Franc, Sauvignon, Syrah and Riesling.

CONCLUSIONS

The preservation of grapevine genetic resources is of prime importance. However, the management of a grapevine germplasm collection is expensive, requiring considerable land area. So, it is important to eliminate repetitions and misclassifications.

The microsatellite analysis was very useful in the identification and discrimination of all the studied cultivars. Investigation of putatively synonymous cultivars has resulted in the confirmation of 17 synonymous. We consider that this work contributed to improve the management of the Grapevine Portuguese Collection. In addition, this set of microsatellite loci will allow for comparisons with genotypes from

any international collection.

Furthermore, the present study will give a good technical support for the update of the list of the cultivars officially authorized for wine production in Portugal (Portaria nº 428/2000, Diário da República nº 163, 17th July 2000), and will also be a support for the trade control of plant material.

The SSR database will be available at the web page of Instituto Nacional de Recursos Biológicos (www.inrb.pt). This database will be regularly updated, what means that new information will be uploaded when necessary.

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REFERENCES

- Almandan M. C., Baleiras-Couto M. M., Pereira H. S., Carneiro L. C., Fevereiro P., Eiras-Dias J. E., Morais-Cecílio L., Viegas W., Veloso M. M. 2007. Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal. *Vitis*, **46**, 116-119.
- Bowers J., Dangl G., Vignani R., Meredith C. 1996. DNA isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* **39**, 628-633.
- Bowers J., Dangl G., Meredith C. 1999. Development and characterization of additional microsatellite DNA markers for grape. *Am. J. Enol. Vitic.* **50**, 243-246.
- Cunha J., Teixeira-Santos M., Veloso M., Carneiro L., Eiras-Dias J. Fevereiro P. 2010. The Portuguese *Vitis vinifera* L. Germplasm: Genetic Relations between Wild and Cultivated Vines. *Ciência e Técnica do Vinho*. 25, 25-37.
- Eiras-Dias J. E., Pereira C. A., Baptista da Cunha J. P. 1988. Catálogo das Castas: Região do Ribatejo, Oeste e Península de Setúbal. Instituto da Vinha e do Vinho. Estação Vitivinícola Nacional.
- Lopes M. S., Santos M. R., Eiras-Dias J. E., Mendonça D., Câmara Machado A. 2006. Discrimination of Portuguese grapevines based on microsatellite markers. *Journal of Biotechnology*, **127**, 34-44.
- Lopes M., Sefc K., Eiras-Dias J., Steinkellner H., Da Câmara Machado A. 1999. The use of microsatellites for germplasm management in a Portuguese grapevine collection. *Theor. Appl. Genet.* **99**, 733-739.
- Sefc K., Regner F., Turetschek E., Glossl J., Steinkellner H. 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome* **42**, 367-373.
- This P., Jung A., Boccaci P., Borrego J., Botta R., Costantini L., Crespan M., Dangl G. S., Eisenheld C., Ferreira-Monteiro F., Grando S., Ibañez 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
- Thomas M., Scott N. 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). *Theor. Appl. Genet.* **86**, 985-990.