Characterisation of the Portuguese grapevine germplasm with 48 single-nucleotide polymorphisms

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Abstract

Background and Aims: Grapevine cultivation has a long tradition in Portugal. Presently, 343 cultivars are legally accepted for wine production. Two hundred and eighty eight accessions from the Portuguese National Ampelographic Collection (including 27 wild grapevines) were genotyped using single-nucleotide polymorphisms (SNP) and compared with the Instituto de Ciencias de la Vid y del Vino-SNP database to reveal identities, synonymies and homonymies.

Methods and Results: A set of 48 SNPs was used to profile the 288 accessions and 263 different genotypes were detected. A comparison with the Instituto de Ciencias de la Vid y del Vino-SNP database unveiled 14 new synonymies among Portuguese, Spanish and other international cultivars and confirmed known synonyms and homonyms. Most of the genotyped cultivars are not cultivated in Spain. Clustering using hierarchical and non-hierarchical methods did not reflect the Portuguese geographical viticulture regions.

Conclusions: New synonyms and homonyms were identified by SNP profiling of the Portuguese grapevine germplasm. Most of the genotyped cultivars are circumscribed to Portugal. Some cultivars are important in the Iberian context.

Significance of the Study: This study confirmed the uniqueness of the Portuguese grapevine genetic pool and disclosed many new synonyms within the cultivars authorised for wine production in Portugal. This study confirmed the suitability of the 48 SNP set for grapevine profiling. Results will help to manage the Portuguese germplasm and to adjust the Portuguese legal framework on the denomination of the cultivars authorised for wine production.

Keywords*: Portuguese germplasm, SNP markers, synonyms and homonyms,* Vitis vinifera *L.*

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated and economically important fruit crops in the world with 7528 Mha of planted vineyards and a global wine production of 252 MhL (Organisation Internationale de la Vigne et du Vin 2013). The Portuguese grapevine-cultivated area is about 177 381 ha, producing 6 MhL of wine (Instituto Nacional de Estatística 2014).

In Portugal 343 grapevine cultivars are legally accepted for wine production (MAMAOT 2012), 240 of them considered being autochthonous and 103 belonging to foreign germplasm. This legal status resulted from a thorough characterisation and analysis of the Portuguese National Ampelographic Collection (PNAC), including the use of morphological descriptors and nuclear microsatellite molecular markers (Almadanim et al. 2007, Veloso et al. 2010, Eiras-Dias et al. 2011, 2013).

The PNAC resulted from a large survey and recollection of grapevine accessions in 1985 when Portugal joined the European Economic Community (today European Union) (Reis 1986). The PNAC also included accessions from regional collections, established by the end of the 19th century after the three major phytopathological problems coming from North America: downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator* Schwein) and phylloxera (*Daktulosphaira vitifoliae*). Some new accessions have been added to the PNAC during recent years, including 63 accessions of *V. vinifera* L. subsp. *sylvestris* (Gmelin) Hegi.

The first known publication referring to cultivar names in Portugal was published in 1532 by Rui Fernandes (Fernandes 1532), in a hand-written description about the city of Lamego, in the Douro region. In 1712, Vicente Alarte published a pioneering Portuguese book about viticulture and oenology in which several grapevine cultivar names and synonyms were mentioned (Alarte 1712). After the establishment of the Porto Wine region in 1756, several publications from the 18th and 19th centuries referred to 865 different grapevine cultivars all over Portugal, but the authors already had conceded the existence of synonyms (Menezes 1896, 1900).

In the Iberian Peninsula, around 1257 Mha are covered with vineyards, and the combined Portuguese and Spanish wine production reaches 36.533 MhL, around 15% of the world wine production (Organisation Internationale de la Vigne et du Vin 2013). The Iberian grapevine germplasm is composed of a large number of unique cultivars (Cabello et al. 2011, Eiras-Dias et al. 2011, 2013). The analysis of diversity with different molecular markers evidenced the uniqueness of the Iberian germplasm (Lopes et al. 1999, Sefc et al. 2000, Ibáñez et al. 2003, Martín et al. 2003, 2006, Almadanim et al. 2007, Veloso et al. 2010, Castro et al. 2011, Zinelabidine et al. 2012), and a hypothesis has been formulated that the

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Iberian grapevine germplasm evolved from a secondary centre of diversity of *V. vinifera* in the Iberian Peninsula (Arroyo-García et al. 2006, Cunha et al. 2009). Wild grapevine populations are well documented in the Iberian Peninsula, and recent studies showed their genetic relationship with autochthonous grapevine cultivars (Cunha et al. 2010, De Andrés et al. 2012). The chlorotype A, specific to Western Europe, is shared by most of the wild grapevines and local cultivars, suggesting domestication events according to Arroyo-García et al. (2006) and Cunha et al. (2010).

Portuguese grapevine diversity has been systematically studied using well-established ampelographic descriptors (Organisation Internationale de la Vigne et du Vin 1983, 2009). The comparison between Portuguese and Spanish cultivars allowed the recognition of several synonyms and homonyms (Eiras-Dias et al. 2011, Ibáñez et al. 2012). They frequently refer to nationally or regionally relevant cultivars for wine production: Tinta Miúda/Graciano; Aragonez or Tinta Roriz/Tempranillo; Jaen/Mencia; Alvarinho/Albariño; and Loureiro/Loureira. Nevertheless, the ampelographic descriptions rely on the training of the ampelographer. In addition, subjectivity of individual observations, as well as growing conditions of the plants, virus status and growing season are further impeding aspects for unambiguous identification by ampelography.

Nuclear microsatellites (nuclear simple sequence repeats) have long proved to be powerful tools for cultivar identification and for diversity (Sefc et al. 2000, Lopes et al. 2006) and parentage studies (Bowers and Meredith 1996, Lacombe et al. 2013). Given its maternal inheritance, chloroplastidial microsatellites (cpSSR) may be used to identify the direction of the crossings, provided that the chlorotypes of the two parents are distinct. The low level of cpSSR polymorphisms result in a lower resolution power for genetic identification, but they are most widely used to assess phylogenetic relationships among plant taxa (Arroyo-García et al. 2002).

Single-nucleotide polymorphisms (SNPs) have been used to determine pedigrees (Ibáñez et al. 2012, Zinelabidine et al. 2012) and to study the genetic structure and domestication history of grape (Myles et al. 2011), but so far have been less used for cultivar discrimination (Dong et al. 2010, Cabezas et al. 2011, Zinelabidine et al. 2014). They are abundant and widespread in most genomes (coding and non-coding regions), and they evolve in a manner well described by simple mutation models (Morin et al. 2004). They are genetically codominant and informative markers for high-resolution genetic maps (Kumar et al. 2009). Compared with nuclear simple sequence repeats, SNPs are easily scored, allowing a more feasible data exchange among laboratories (Lijavetzky et al. 2007).

We used the 48 SNP set developed by Cabezas et al. (2011) to genotype the Portuguese autochthonous cultivars and compare them with the Instituto de Ciencias de la Vid y del Vino (ICVV)-SNP database. This set of 48 stable SNP markers has a high discrimination power, a uniform genome distribution and has been proposed as a standard set for *V. vinifera* L. genotyping (Cabezas et al. 2011). With this work, we aimed: (i) to accurately discriminate among the Portuguese autochthonous cultivars; (ii) to detect new synonyms and homonyms in the Portuguese grapevine germplasm and to confirm those already described; and (iii) to increase our knowledge on the genetic relationships among Portuguese cultivars and wild grapevines. Preservation and management of the Portuguese and Iberian *V. vinifera* germplasm will benefit with the clarification provided by this work.

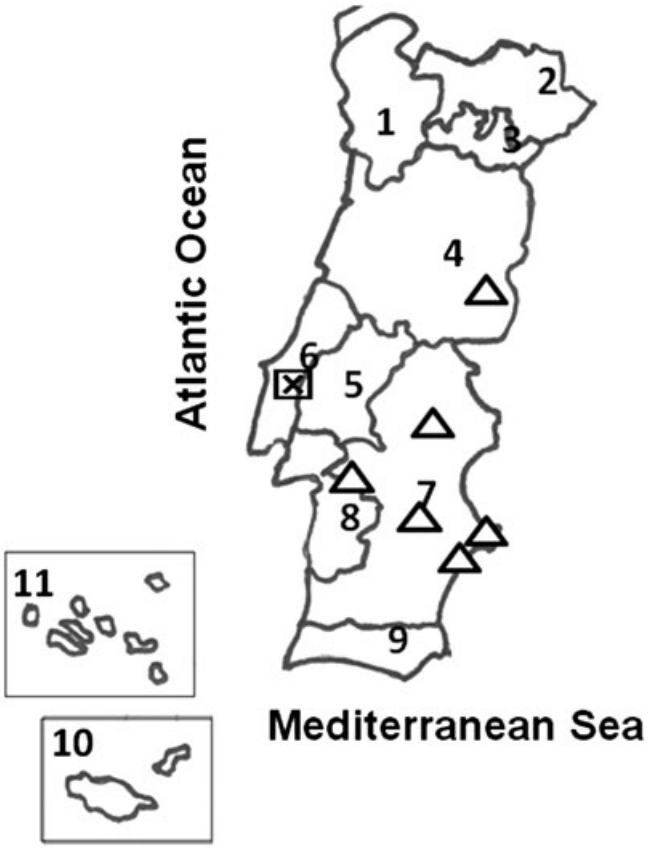

Figure 1. Map of Portuguese wine regions: 1, Vinhos Verdes; 2, Trás-os-Montes; 3, Douro and Porto; 4, Beiras; 5, Tejo; 6, Lisboa; 7, Alentejo; 8, Península de Setúbal; 9, Algarve; 10, Madeira; and 11, Açores. All accessions studied were identified to the correspondent Portuguese wine regions in Table 1 where the plant material was collected initially in field, including regional collections. Location of Portuguese National Ampelographic Collection (PNAC) (⊠); location of wild populations in situ (△).

Materials and methods

Plant material sampling

Two hundred eighty eight accessions of *V. vinifera* L. (Table 1, Figure 1) were collected at the PNAC (PNAC-PRT051). These 288 accessions included 239 non-redundant autochthonous grape cultivars, 27 wild grapevines, three unknown cultivars, ten cultivars to test the methodology and nine cultivars with great relevance to Portugal. The remaining 104 cultivars legally accepted in Portugal for wine production (MAMAOT 2012) are internationally known cultivars and for that reason were not included in this study. The PNAC-PRT051 collection was established in 1988 and is located at Quinta da Almoinha, Dois Portos, Torres Vedras [39°02′34.03″N, 9°10′57.41″W – Figure 1 (⊠)]. This collection is managed by the Instituto Nacional de Investigação Agrária e Veterinária, from the Portuguese Ministry of Agriculture and is the reference collection for cultivars allowed in Portugal for wine production (MAMAOT 2012).

Extraction of DNA

Young leaves were collected and frozen at −80°C, and DNA was extracted as described by Thomas et al. (1993), with minor modifications. The quality and concentration of the DNA were determined in agarose (0.8%) stained with ethidium bromide and visualised on a UV transilluminator. Concentration was calculated by comparing with known DNA concentration (50, 100 and 200 ng/μL) of λDNA (HindIII Fragments, 0.1 μg/μL, Invitrogen, Carlsbad, CA USA). Final concentration was

Table 1. The Portuguese accessions studied in this work.

Accession name	Code†	Cultivar name‡	Berry§	Origin¶
Agronómica	41505	Agronómica	N	6
Água Santa	50615	Água Santa	N	6
Alfrocheiro Preto	52003	Alfrocheiro	N	4
Alicante Branco	50711	Alicante Branco	B	6
Almafra	52313	Almafra	B	6
Alvadurão	52114	Alvadurão	B	6
Alvarelhão	53207	Alvarelhão	N	4
Alvarelhão Ceitão	41209	Alvarelhão Ceitão	N	3
Alvarinho	52007	Alvarinho	B	1
Alvarinho Lilaz	40701	Alvarinho Lilás	B	6
Azal Tinto	52908	Amaral	N	5
Amor-Não-Me-Deixes	51003	Amor Não Me Deixes	N	7
Preto Martinho	53204	Amostrinha	N	6
Antão Vaz	52316	Antão Vaz	B	7
Aragonez	52603	Aragonez	N	7
Arinto	52311	Arinto	B	6
Arinto do Douro	51412	Arinto do Interior	B	4
Arinto do Pico	50217	Arinto dos Açores	B	11
Arjunção	52104	Arjunção	N	9
Assaraky	40404	Assaraky	B	6
Avesso	52310	Avesso	B	1
Azal	52809	Azal	B	1
Baga	52606	Baga	N	4
Barcelo	52407	Barcelo	B	4
Barreto de Semente	41302	Barreto	N	3
Bastardo	52803	Bastardo	N	8
Bastardo Branca	51117	Bastardo Branco	B	3
Bastardo Roxo	41708	Bastardo Roxo	Rs	3
Batoca	52507	Batoca	B	1
Beba	51808	Beba	B	9
Bical	52016	Bical	B	6
Boal Branco	52116	Boal Branco	B	6
Boal Espinho	52017	Boal Espinho	B	6
Monvedro de Sines	41601	Bonvedro	N	8
Borraçal	52807	Borraçal	N	1
Anadia Branca	50314	Branca de Anadia	B	6
Branco Desconhecido	41107	Branco Desconhecido	Rs	3
Branco Especial	51216	Branco Especial	B	3
Branco Gouvães	41105	Branco Gouvães	B	3
Branco Guimarães	51018	Branco Guimarães	B	3
Branco Valente	40502	Branco Valente	B	3
Dona Branca	52117	Branda	B	4
Branjo	41202	Branjo	N	1
Cabinda	53103	Cabinda	N	6
Camarate	52402	Camarate	N	6
Campanário	41806	Campanário	N	6
Caracol	50914	Caracol	B	10
Caramela	51016	Caramela	B	3
Carrasquenho	52605	Carrasquenho	N	6
Carrega Branco	51816	Carrega Branco	B	3
Carrega Burros	52902	Carrega Burros	N	5
Cascal	51517	Cascal	B	1
Casculho	50901	Casculho	N	3
Castália	40702	Castália	B	6
Castelã	51002	Castelã	N	3
Castelão Francês	53106	Castelão	N	5
Castelão Branco	52615	Castelão Branco	B	6
Castelino	52706	Castelino	N	6
Castelo Branco	50309	Castelo Branco	B	6
Casteloa	41303	Casteloa	N	3
Cerceal Branco	52410	Cerceal Branco	B	3
Cercial	52412	Cercial	B	4
Tinta de Cidadelhe	51308	Cidadelhe	N	3
Cidreiro	51404	Cidreiro	N	4
Côdega de Larinho	51317	Códega do Larinho	B	3
Complexa	50201	Complexa	N	6
Concieira	50902	Concieira	N	3
Coração de Galo	51304	Coração de Galo	N	4
Cornichon	40708	Cornichon	B	7
Cornifesto	52004	Cornifesto	N	3
Corropio	51405	Corropio	N	7
Corvo	51207	Corvo	N	6
Dedo de Dama	51209	Dedo de Dama	B	6
Deliciosa	41707	Deliciosa	N	6
Boal Durão	50818	Diagalves	B	4
Diagalves	52513	Diagalves	B	6
Doçal	50904	Doçal	N	1
Doçal de Refoios	50905	Doce	N	3
Dona Joaquina	51609	Dona Joaquina	B	6
Donzelinho Branco	52307	Donzelinho Branco	B	3
Donzelinho Roxo	41709	Donzelinho Roxo	Rs	3
Donzelinho Tinto	52306	Donzelinho Tinto	N	3
Arinto	51411	Dorinto	B	3

Table 1. (continued)

Accession name	Code†	Cultivar name‡	Berry§	Origin¶
Alfrocheiro Branco	51610	Douradinha	B	3
Douradinha	51410	Douradinha	B	4
Encruzado	52207	Encruzado	B	4
Tinta Engomada	51008	Engomada	N	3
Esganinho	41103	Esganinho	B	1
Esganoso de P^{te}de Lima	50915	Esganoso	B	1
Espadeiro	52904	Espadeiro	N	1
Espadeiro Mole	51604	Espadeiro Mole	N	1
Estreito Macio	51017	Estreito Macio	B	3
Alentejana	41502	Fepiro	N	6
Fernão Pires	52810	Fernão Pires	B	6
Fernão Pires Rosado	52815	Fernão Pires Rosado	Rs	5
Ferral	50104	Ferral	Rg	6
Folgasão	52709	Folgasão	B	3
Folgasão Roxo	52708	Folgasão Roxo	R	4
Folha de Figueira	51514	Folha de Figueira	B	3
Fonte Cal	52314	Fonte Cal	B	4
Galego	41203	Galego	N	1
Galego Dourado	52913	Galego Dourado	B	6
Generosa	40808	Generosa	B	6
Gonçalo Pires	50802	Gonçalo Pires	N	3
Gouveio	52112	Gouveio	B	3
Gouveio Estimado	50617	Gouveio Estimado	B	3
Gouveio Preto	41305	Gouveio Preto	N	3
Gouveio Real	50616	Gouveio Real	B	3
Gouveio Roxo	41702	Gouveio Roxo	Rs	3
Grangeal	51602	Grangeal	N	3
Granho	40606	Granho	B	7
Jaen	52503	Jaen	N	4
Jampal	52515	Jampal	B	6
Labrusco	41204	Labrusco	N	1
Lameiro	50611	Lameiro	B	1
Larião	51113	Larião	B	7
Listrão Roxo	41605	Listrão	Rs	10
Loureiro	52213	Loureiro	B	1
Lourela	50708	Lourela	N	3
Lusitano 7	41503	Lusitano	N	6
Luzídio	51115	Luzidio	B	4
Tinta Malandra	50608	Malandra	N	3
Malvarisco	53308	Malvarisco	N	8
Malvasia	52714	Malvasia	B	6
Malvasia Babosa	40603	Malvasia Babosa	B	10
Malvasia Cabral	51212	Malvasia Cabral	Rs	3
Malvasia B de S. Jorge	40604	Malvasia de São Jorge	B	10
Malvasia Fina	52512	Malvasia Fina	B	3
Assario Roxo	52612	Malvasia Fina Roxa	Rs	4
Farinheira	41304	Malvasia Parda	B	3
Malvasia Preta	53205	Malvasia Preta	N	3
Pinheira Roxa	41703	Malvasia Preta Roxa	Rs	3
Malvasia Rei	53013	Malvasia Rei	B	3
Malvasia	50912	Malvasia-Branca	B	11
Malvasia de Oeiras	40704	Malvoeira	B	6
Manteúdo	51413	Manteúdo	B	7
Manteúdo Preto	41603	Manteú do Preto	N	7
Marquinhas	53312	Marquinhas	B	6
Marufo	52002	Marufo	N	4
Melhorio	41205	Melhorio	N	1
Tinta Melra	41309	Melra	N	3
Mindelo	41607	Mindelo	N	6
Mondet	50702	Mondet	N	3
Monvedro	51804	Monvedro	N	4
Moreto	52301	Moreto	N	7
Moscadet	51417	Moscadet	B	3
Portalegre	41508	Moscargo	N	6
Moscatel Galego	52915	Moscatel Galego Branco	B	3
Moscatel Galego T	41301	Moscatel Galego Tinto	N	3
Moscatel Branco	53015	Moscatel Nunes	B	8
Mourisco	51701	Mourisco	N	1
Mourisco Branco	50916	Mourisco Branco	B	3
Mourisco de Semente	51402	Mourisco de Semente	N	3
Mourisco de Trevões	41306	Mourisco de Trevões	N	3
Mulata	53407	Mulata	N	6
Naia	40703	Naia	B	6
Negra Mole	52202	Negra Mole	N	9
Nevoeira	52005	Nevoeira	N	3
Padeiro de Basto	50806	Padeiro	N	1
Parreira Matias	52702	Parreira Matias	N	6
Patorra	52006	Patorra	N	3
Pé Comprido	41002	Pé Comprido	B	3
Pedral	52105	Pedral	N	1
Perrum	51617	Perrum	B	9
Pical-Polho	51007	Pical	N	1
Tourigo do Douro	51606	Pilongo	N	4

Table 1. (continued)

Accession name	Code†	Cultivar name‡	Berry§	Origin¶
Branco Escola	51217	Pintosa	B	1
Português Azul	50605	Português Azul	N	3
Praça	51715	Praça	B	3
Preto Cardana	52705	Preto Cardana	N	5
Preto Martinho	51803	Preto Martinho	N	3
Primavera	53102	Primavera	N	6
Promissão Branca	40501	Promissão	B	3
Rabigato	52014	Rabigato	B	3
Rabigato Francês	51613	Rabigato Franco	Rs	3
Rabigato Moreno	50917	Rabigato Moreno	B	3
Rabo de Ovelha Tinto	52903	Rabo de Anho	N	1
Rabo de Ovelha	52011	Rabo de Ovelha	B	6
Ramisco	52203	Ramisco	N	6
Boal Ratinho	52309	Ratinho	B	6
Tinta Ricoca	51103	Ricoca	N	3
Rio Grande	40809	Rio Grande	B	6
Roal	53806	Roal	Rs	8
Tinta do Rodo	51708	Rodo	N	3
Tinta Roseira	50707	Roseira	N	3
Roupeiro	51314	Roupeiro Branco	B	6
Roxo de Vila Flôr	41705	Roxo Flor	R	3
Roxo Rei	50918	Roxo Rei	R	3
Rufete	52106	Rufete	N	4
Budelho	40707	Samarrinho	B	7
Samarrinho	51516	Samarrinho	B	3
Santarém	52304	Santareno	N	3
São Mamede	51611	São Mamede	B	1
Sarigo	51316	Sarigo	B	3
Seara Nova	40403	Seara Nova	B	6
Sercial	40505	Sercial	B	10
Sercialinho	51011	Sercialinho	B	6
Sevilhão	51403	Sevilhão	N	3
Sousão	51901	Sezão	N	1
Sabro	51213	Síria	B	9
Síria	51914	Síria	B	4
Molinho do vau	51911	Tamarez	B	6
Tamarez	51910	Tamarez	B	5
Teinturier	53807	Teinturier	N	6
Terrantez	52210	Terrantez	B	4
Terrantez	50216	Terrantez do Pico	B	11
Tinta Aguiar	50703	Tinta Aguiar	N	3
Tinta do Aurélio	40609	Tinta Aurélio	N	3
Tinta Barroca	52905	Tinta Barroca	N	3
Tinta Caiada	51905	Tinta Caiada	N	7
Tinta Carvalha	52201	Tinta Carvalha	N	3
Tinta da Barca	52101	Tinta da Barca	N	3
Tinta de Alcobaça	41504	Tinta de Alcobaça	N	6
Bastardo Espanhol	51108	Tinta de Lisboa	N	4
Tinta Miúda	50706	Tinta Fontes	N	3
Tinta Francisca	52502	Tinta Francisca	N	3
Tinta Gorda	50607	Tinta Gorda	N	3
Tinta Grossa	52906	Tinta Grossa	N	7
Tinta Martins	50602	Tinta Martins	N	3
Tinta Mesquita	50604	Tinta Mesquita	N	3
Rabo de Ovelha Tinto	51703	Tinta Negra [6]	N	4
Saborinho	51711	Tinta Negra [6]	N	11
Tinta Negra Mole	51202	Tinta Negra [6]	N	10
Tinta Roriz	51208	Tinta Penajoia	N	3
Tinta Penajóia	50907	Tinta Pereira	N	3
Tinta Pereira	50807	Tinta Pomar	N	3
Tinta Pomar	51307	Tinta Tabuaço	N	3
Tinta de Tabuaço	52505	Tintem	N	6
Benfica	51205	Tintinha	N	7
Tintinha	53307	Tinto Cão	N	3
Tinto Cão	52506	Tinto Pegões	N	6
Tinto Pegões	50705	Touriga Fêmea	N	3
Touriga Brasileira	52205	Touriga Franca	N	3
Touriga Francesa	52206	Touriga Nacional	N	3
Touriga Nacional	52710	Trajadura	B	1
Trajadura	41206	Transâncora	N	1
Transancora	41510	Triunfo	N	5
Triunfo	50909	Trigueira	Rs	3
Malvasia Trigueira	41602	Trincadeira	N	7
Folha de Abóbora	53006	Trincadeira	N	5
Trincadeira Preta	51012	Trincadeira Branca	B	6
Trincadeira Branca				

Table 1. (continued)

Accession name	Code†	Cultivar name‡	Berry§	Origin¶
Trincadeira das Pratas	52216	Trincadeira das Pratas	B	5
Uva Cão	51415	Uva Cão	B	4
Uva Cavaco	51211	Uva Cavaco	B	4
Uva Salsa	50311	Uva Salsa	B	6
Valbom	53206	Valbom	N	6
Tinta Valdosa	51608	Valdosa	N	3
Tinta Varejoa	50808	Varejoa	N	3
Boal Vencedor	52111	Vencedor	B	6
Verdelho	50317	Verdelho	B	11
Verdelho	51509	Verdelho	B	10
Verdelho Roxo	51513	Verdelho Roxo	Rs	11
Verdelho Tinto	51806	Verdelho Tinto	N	1
Verdial Douro	41208	Verdial Tinto	N	3
Verdial Vinhos Verdes	41207		N	1
Vinhão	51902	Vinhão	N	1
Viosinho	52715	Viosinho	B	3
Vital	52614	Vital	B	6
Unknown TB	62413		N	3
Unknown MRT	62513		B	3
Unknown 4, BS	41509		B	5
V sylvestris	110501			8
V sylvestris	110504			8
V sylvestris	110602			8
V sylvestris	110603			8
V sylvestris	110303			4
V sylvestris	110307			4
V sylvestris	110402			4
V sylvestris	110403			4
V sylvestris	110405			4
V sylvestris	120305			8
V sylvestris	120307			8
V sylvestris	120309			8
V sylvestris	120404			8
V sylvestris	120501			8
V sylvestris	110102			7
V sylvestris	110105			7
V sylvestris	110201			7
V sylvestris	120104			7
V sylvestris	120106			7
V sylvestris	120108			7
V sylvestris	120109			7
V sylvestris	120201			7
V sylvestris	120203			7
V sylvestris	120205			7
V sylvestris	120207			7
V sylvestris	120209			7
V sylvestris	120302			7

The colour of the berries is indicated according to Organisation Internationale de la Vigne et du Vin recommendations as N (noir – blue), B (blanc – white) Rg (rouge – red) and Rs (rose). †code of PNAC (PRT051) in field collection; ‡legal cultivar name in Portugal; §berry colour; ¶geographical origin (region where plant material was collected) in Portugal (Figure 1, wine region) of the accession.

confirmed using a NanoDrop 2000 C UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA)

Analysis with SNPs

A set of 48 nuclear SNPs was used as described in Cabezas et al. (2011); the SNP genotyping was carried out at the Centro Nacional de Genotipado (http://www.cegen.org) in Spain using the SNPlex (Applied Biosystems, Waltham, MA USA) or Veracode (Illumina, San Diego, CA, USA) technologies according to Zinelabidine et al. (2012, 2015) .

Data analysis

The SNP profiles obtained were compared using the Excel Microsatellite Toolkit (Park 2001) to determine the matching accessions and to select the non-redundant genotypes existing in the Portuguese set of accessions. A limit of 30% missing data was considered for comparison of profiles.

The Portuguese non-redundant SNP genotypes were compared with the ICVV-SNP database to determine identities, synonyms, homonyms and mistakes. The ICVV-SNP database accounts for 1761 unique genotypes, 398 of them from *sylvestris* vines. The ICVV-SNP includes most of the *V. vinifera* genotypes from the collection of the Instituto de Ciencias de la Vid y del Vino, in Logroño, Spain (ICVV, ESP217) and from the Spanish reference grapevine collection, in El Encín [Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), ESP080]. In addition, it also contains genotypes from several origins: Algeria, Argentina, Australia, Belgium, Chile, France, Iran, Italy, Montenegro, Morocco, Portugal, Romania, Spain and Tunisia.

The GenAlex v6.501 program package (Peakall and Smouse 2012) was used to calculate the genetic distances among the different Portuguese genotypes, for a principal coordinate analysis (PCoA), and to test population assignment considering wild and cultivated populations. Missing data were assigned as −9 as instructed by the program package.

The software MEKA 5.2 (http://www.megasoftware.net) was used to construct the phenetic tree based on the unweighted pair-group method using arithmetic averages (UPGMA) over a matrix of genetic distances obtained with the simple match algorithm (Tamura et al. 2011).

All accesses used in this work are identified by the number and the prime name of the *Vitis* International Catalogue of Varieties (*VIVC*, www.vivc.de, accessed February 2015).

Results and discussion

The 48 SNP set provided genotypes for all of the 288 accessions. This SNP panel has a discrimination power identical to 15 simple sequence repeat (SSR) markers, as stated by Cabezas et al. (2011). In this work, the non-redundant genotypes are distinguished by at least six different alleles, for the *sylvestris* plants, and at least nine different alleles for the grapevine cultivars. The rate of missing data was 8.1%, which is considered low. Fifty-six accessions were profiled with 100% of the SNPs, and 261 accessions were profiled with at least 35 SNPs. Two accessions [Trigueira (PRT051-50909) and Folha de Figueira (PRT051-51514)] were profiled with only 34 SNPs (Table S1). The low rate of missing data further supports the proposal of Cabezas et al. (2011) to consider this panel as a standard for grapevine identification, when using SNP profiling.

Cultivar identification and detection of synonymies, homonymies and misnames

Twenty-five redundant genotypes were found among the 288 accessions. Comparison among samples assisted in the

identification of three unknown accessions (Unknown TB_ PRT051-62413; Unknown 4 BS_ PRT051-41509; Unknown MRT_PRT051-62513), recently incorporated in the PNAC (Table 1). These accessions were identified as Cornifesto (PRT051-62413), Síria (PRT051-41509) (Doña Blanca) and Rabigato (PRT051-62513). These identifications were confirmed after careful re-analysis of the morphological descriptions undertaken in 2014 (data not shown).

Two accessions labelled as Mindelo (PRT051-41607) and Triunfo (PRT051-41510) (Table 1) bore the same genotype (44/48 SNP scored). These two cultivars are legally accepted for wine production (MAMAOT 2012) and have been considered as distinct, further supported by the SSRs profiling in Veloso et al. (2010). Ampelographic verification showed that the two PNAC accessions share common traits. Further analysis of other accessions of Mindelo and Triunfo are needed to clarify this situation (misname or synonym), because no other matches were found in the ICVV-SNP database. Likewise, Verdial (PRT051-41207) and Melhorio (PRT051-41205) are two accessions from the Vinhos Verdes region that shared the same SNP profile (43/48 SNP scored) and are probably synonyms (Table 2). Melhorio is an old minor cultivar restricted to the Basto subregion of Vinhos Verdes, the SSR profile of which has been already published (Castro et al. 2012).

Two hundred and sixty-three non-redundant genotypes were found, 236 of them corresponding to cultivars that are authorised for wine production in different Portuguese wine regions (Table 1, Figure 1), all of them listed in the *V*IVC database (Table S1). The remaining 27 non-redundant genotypes correspond to wild grapevines maintained at PNAC, which were originally collected in six wild populations, located in three different hydrological basins (Tejo, Guadiana and Sado), in the south of Portugal (Figure 1).

A large number of unique genotypes (134 cultivars plus 27 wild grapevines) did not match any of the 1761 existing genotypes in the ICVV-SNP database, indicating that they are likely autochthonous from Portugal. All these correspond to minor cultivars and most of them are, so far, only known in Portuguese grapevine collections. The other 102 genotypes matched with existing genotypes in the ICVV-SNP database († in Table S1). Most of the matches (88) corresponded to accessions with the same or similar names, or with names corresponding to known synonyms, but 14 new putative synonyms were found (Table 2).

Alvarelhão Ceitão (PRT051-41209) and Tinta Castellõa (ESP217-5316) showed identical SNP profiles (46/48 SNPs scored) and are synonyms (Table 2). Although these cultivars were not known as being synonymous, the names Alvarelhão, Ceitão and Tinta Castellôa (syn. Castellã) are referred as black-berried cultivars from the Douro (Figure 1, region 3) in Portuguese historical written records (Menezes 1896). In Portugal there is a partial homonym (Menezes 1896, MAMAOT 2012) of a non-related black grape cultivar named Alvarelhão (PRT051-53207; *V*IVC_ 1650).

Arjunção (PRT051-52104; *V*IVC_17358) has the same SNP profile as Listan Prieto (*V*IVC_6860) (48/48 SNPs scored). Comparing SSR profiles from the *V*IVC database and from Veloso et al. (2010), we observed a 4 bp difference in the VVS2 SSR marker. This cultivar is mentioned for the first time in Portugal by Menezes (1900) under the name 'Argenção' as a red cultivar from Algarve (Figure 1, region 9). The analysed sample was originally collected at Portimão, in the Algarve region (Table 1). Arjunção and Listan Prieto are thus synonymies not currently listed in *V*IVC database.

Batoca (PRT051-52507; *V*IVC_1037) from the Vinhos Verdes region and Blanca Mantilla (MBG-89) from Galicia (Spain) have identical SNP profiles (41/48 SNPs scored). This synonym could be confirmed using published SSR data [www.vitis.mbg.csic.es/vitis/es/variedad.php?id=11, Veloso et al. (2010)]. The French Network of Grapevine Repositories database from INRA Vassal-Montpellier identified this synonym after the entry of Blanca Mantilla of Misión Biológica de Galicia (MBG) in its collection, in 2011 (http://bioweb.supagro.inra.fr/collections_vigne). Galicia is an autonomous region of Spain, which borders the Vinhos Verdes and the Trás-os-Montes wine regions in the North of Portugal (Figure 1, regions 1 and 2). Until the first half of the 20th century, Batoca spread throughout Portugal, which explains why 12 of the 15 synonyms in the *V*IVC database have Portuguese names. On

Table 2. Matching single-nucleotide polymorphism genotypes (possible new synonyms) identified between Portuguese, Spanish and other international cultivars.

Cultivar name		Synonyms		Recommended *V*IVC name and number	
Cultivar name (PRT051 code)	*V*IVC No.	Cultivar name (code)	*V*IVC No.	Prime name or prime name candidate	*V*IVC No.
Alvarelhão Ceitão (41209)	368	Tinta Castellõa (ESP217-5316)		Alvarelhão Ceitão	368
Arjunção (52104)	17358	Listan Prieto	6860	Listan Prieto	6860
Batoca (52507)	1037	Blanca Mantilla (MBG-89)		Batoca	1037
Caracol (50914)	17664	Cedrés 23.2 (ICIA)		Caracol	17664
Carrega Branco (51816)	2124	Chavacana; Colgadeira (MBG)		Carrega Branco	2124
Castelino (52706)	17259	Corvo (PRT051_51207) Auban (ESP217-5015)		Aubun	761
Malvasia Babosa (40603)	14139	Malmsey (ESP080-BGVCAM2259)		Malvasia Babosa	14139
Malvasia-Branca (50912)	23162	Gros Vert Blanc	5082	Gros Vert Blanc	5082
Moscadet (51417)	15679	Meslier Saint Francois	7677	Meslier Saint Francois	7677
Perrum (51617)	9183	Torrontés (ESP217-5194); Turruntes (ESP217-6009, ESP217-6011)		Perrum Branco	9183
Pintosa (51217)	9290	Espadeiro (ESP217-5063)		Branco Escola	9290
Roal (53806)	10298	Rocia (ESP080- BGVCAM2055)	40057	Roal	10298
Santareno (52304)	40705	Etraire de la Dui	3993	Etraire de la Dui	3993
Verdial, VV (41207)		Melhorio (PRT051_41205)	17255	Melhorio	17255

*V*IVC, *Vitis* International Variety Catalogue.

the contrary, Blanca Mantilla does not appear in the *V*IVC database. The oldest written reference to Batoca places its origin in the Basto region (Eastern part of wine area 1) in the north of Portugal (Lacerda Lobo 1790). Today it is one of the seven most important white cultivars of the Vinhos Verdes wine region.

Caracol (PRT051-50914; *V*IVC_17664) and Cedrés [Instituto Canario de Investigaciones Agrarias (ICIA), Canary Islands, Spain] share the same SNP profiles (38/48 SNPs scored). Caracol is a white cultivar from the Porto Santo Island (Figure 1, region 10) in the Madeira Islands archipelago, 400 km above the Canary Islands. It has two synonyms in the *V*IVC, but none of them is Cedrés, which is not present in the *V*IVC database. This previously unknown synonymy was also confirmed by comparing the SSR profiles obtained by Veloso et al. (2010) and Hernández Ferrer (Rodríguez-Torres 2013).

Carrega Branco (PRT051-51816; *V*IVC_2124) shares the same SNP profile with Chavacana and Colgadera (MBG) from Galicia (48/48 SNPs scored). These synonyms could be confirmed analysing the SSR data of Carrega Branco obtained by Veloso et al. (2010) (from PRT051) and Colgadera by Vilanova et al. (2009) (from the MBG). Chavacana and Colgadeira are new synonyms of Carrega Branco, to be added to the seven already disclosed by *V*IVC. Fernández-González et al. (2007) achieved a different SSR profile for Colgadera but this was sampled in the field in Castilla-La Mancha region and revealed to be Bobal (*V*IVC_1493) and is probably a homonymy. The presence of Carrega Branca in the Douro region in 1877 (Marques Loureiro) is historically cited by Menezes (1896).

Castelino (PRT051-52706) in Portugal and the French cultivar Aubun (ESP217-5015) had been previously identified as synonyms (Maul and Töpfer 2015) (*V*IVC_761) (44/48 SNP scored). Corvo (PRT051-51207) was identified by SNP analysis as a new synonym of those two cultivars. Although the morphological data foresaw this synonymy, Veloso et al. (2010) were not able to verify it, using six SSR loci. In Portugal, this cultivar is almost extinct and was usually found in the Lisbon wine region (Figure 1, region 6).

The cultivar Malvasia Babosa (PRT051-40603; *V*IVC_14139) shares the same SNP profile with Malmsey (ESP080-BGVCAM2259) (48/48 SNP scored). Malvasia Babosa is a cultivar restricted to Madeira Island where it has been reported since 1879 (Garcia Ramos cited in Menezes 1896) and historically has been used in the production of Madeira wine (Figure 1, region 10). Malmsey is a common name attributed to the sweet Madeira wine, which would explain the name of the accession conserved in Spain, where it is not cultivated and where it was introduced from the USA (www.madrid.org/coleccionvidencin/index). Malvasia is a widespread denomination used in 217 distinct cultivars listed in the *V*IVC database. The Portuguese list of grapevines for wine production contains 15 different white, rose and black cultivars with Malvasia as part of its name (MAMAOT 2012).

Malvasia Branca (PRT051-50912; *V*IVC_23162) and Gros Vert Blanc (*V*IVC_5082) also shared the same SNP genotype (43/48 SNPs scored). When comparing the *V*IVC SSR profiles of Gros Vert Blanc and our SSR data of Malvasia Branca (VVMD25 241:257; VVMD28 237:251; VVMD32 241:273; VVMD5 224:236; VVMD7 243:249; VVS2 135:139; VrZAG62 188:200; VrZAG79 255:257, unpublished), we found that eight of the loci have identical alleles, while VVMD27 is homozygous (179:179) in our accession but heterozygous in the *V*IVC (180:195). Gros Vert Blanc is a grape cultivar from France and Malvasia Branca (PRT051-50912) was originally collected in the Azores Islands. No references could be found on the introduction of this cultivar in the Azores.

Moscadet (PRT051-51417; *V*IVC_15679) and Meslier Saint François (VIVC_7677) share the same SNP genotype (48/48 SNPs scored). When comparing the SSR profile obtained for Meslier Saint-Francois (FRA139-397) by Lacombe et al. (2013) with our SSR data of Moscadet (VVMD25 241:243; VVMD27 175:179; VVMD28 231:237; VVMD32 273:273; VVMD5 230:232; VVMD7 239:239; VVS2 135:135; VrZAG62 188:196; VrZAG79 237:247, unpublished), we found seven of the loci sharing the same alleles.

Perrum (PRT051-51617) is the official Portuguese name (MAMAOT 2012) of a white cultivar with the prime name Perrum Branco (*V*IVC_9183). Perrum has the same SNP profile as the cultivar Torrontes/Turruntes (ESP217-5194/ESP217-6009 and ESP217-6011) (43/48 SNPs scored) collected in La Rioja and maintained in the ICVV collection (Spain). Nevertheless the comparison of the SSR profile of Perrum (Veloso et al. 2010) with all other different Torrontes and Turruntes profiled accessions in the Spanish collections (Borrego et al. 2002, Martín et al. 2003, 2006, Gago et al. 2009) show that they are all different. This is a clear case of multiple homonymies, and more cultivars with the same name can be found when considering accessions from Galicia (Spain, www.vitis.mbg.csic.es/variedad.php?id=53) and Argentina (Agüero et al. 2003). Perrum and the Spanish cultivar Perruno (*V*IVC_9185) also bear different SSR profiles. In the ICVV-SNP database, there is another accession named Perrum (ESP217-5146), deposited by J. Böhm from Viveiros Plansel (Portugal), which has a different SNP profile, probably being again a case of homonymy. The Galician cultivar Torrontés is the Portuguese cultivar Malvasia Fina (*V*IVC_715).

Pintosa (PRT051-51217) is the official Portuguese name (MAMAOT 2012) of a white cultivar with the prime name Branco Escola (*V*IVC_9290). Pintosa has the same SNP profile as the cultivar Espadeiro maintained in the ICVV collection (ESP217-5063) (43/48 SNPs scored). In Portugal, there are two other different cultivars named Espadeiro: Espadeiro (*V*IVC_24552, prime name Espadeiro Tinto) and Espadeiro Mole (*V*IVC_7340, prime name Manseng Noir), both black cultivars from the Vinhos Verdes region. The black cultivar Trincadeira (*V*IVC_15685) used for the production of Carcavelos wine (Figure 1, region 6) is also known as Espadeiro. The SNP profiles as well as the SSR profiles (Veloso et al. 2010) of Pintosa and the two black Espadeiro and Espadeiro Mole cultivars are different, excluding the possibility of being originated by somatic variation for the berry colour. Both Lacerda Lobo (1790) and Villa Maior (1866) referred to an Espadeiro Branco cultivar in the Vinhos Verdes wine region but by the late 20th century this name was lost (Instituto da Vinha e do Vinho 1991). We could not conclude that Pintosa is the ancient Espadeiro Branco. Espadeiro is a Portuguese word meaning swordsman or sword maker and is frequently used in viticulture to describe an erect shoot habit (Organisation Internationale de la Vigne et du Vin 2009, descriptor No. 006, note 1), which resulted in its spreading in cultivars names.

Roal (PRT051-53806; *V*IVC_10298) and Rocia (ESP080-BGVCAM2055) share an identical SNP profile (46/48 SNPs scored), and its synonymy could be confirmed by comparing the SSR profiles in Veloso et al. (2010) and Martín et al. (2003). Roal is a rose cultivar from Península de Setúbal region (Figure 1, region 8) and was planted in the past using a traditional pergola training system near farmhouses. This synonymy was mentioned in the classical work of Viala and Vermorel (1905–1910).

Santareno (PRT051-52304; *V*IVC_40705) and Etraire de la Dui (*V*IVC_3993) share the same SNP profile (48/48 SNPs scored). When comparing the SSR profile for Etraire de la Dui (Pl@ntGrape 2007. http://plantgrape.plantnet-project.org) and

our own SSR genotyping of Santareno (VVMD25 240:250; VVMD27 179:189; VVMD32 241:241; VVMD5 228:234; VVMD7 240:244; VVS2 135:153; VRZAG62 187:193; VRZAG79 251:255, unpublished), it was found that both cultivars share the same alleles in eight loci, but show different allels for the VVMD28 locus (Santareno – 237:269; Etraire de la Dui - 233:267).

Genotyping with SNPs is also useful to solve questions regarding possible incorrect names, synonyms or homonyms. Amaral (PRT051-52908) and Melhorio (PRT051-41205), two cultivars from Vinhos Verdes (Figure 1, region 1), are difficult to distinguish morphologically. This led to mixtures in the nurseries. The SNP genotypes of Amaral (44/48 SNPs scored) and Melhorio (43/48 SNPs scored) confirmed that they are different cultivars, as previously determined by Castro et al. (2011) using 12 SSR.

Even though they are clearly distinguishable by morphologic traits, Gouveio (PRT051-52112) from Dão (Figure 1, part of region 4) and Verdelho (PRT051-50317) from Madeira and Azores Islands (Figure 1, regions 10 and 11) are a case of confounded identity, because in the Dão wine region Gouveio is also known as Verdelho, a clear case of homonymy. This led to many mistakes in nurseries that could be corrected with SSR profiling (Almadanim et al. 2007) and now with SNP profiling.

The accessions Saborinho (PRT051-51711) from the Azores Islands, Tinta Negra Mole (PRT051-51202) from Madeira Island, Rabo de Ovelha Tinto (PRT051-51703) from Pinhel and Molar (PRT051-52703) from Colares (not included in this study) were previously identified as synonyms when genotyped with SSR (Veloso et al. 2010). The first three accessions were also genotyped with SNP, confirming the results obtained through SSR genotyping. This cultivar is clearly an ancient one, explaining the existence of multiple synonyms (including the ancient Portuguese spelling Mollar, similar to that adopted by *V*IVC, Molar). This cultivar has no clear geographical origin in Portugal, being cultivated since the 18th century in the wine regions of Pinhel, Colares and Madeira (Figure 1, regions 4, 6 and 10). In Spain, this cultivar is known as Bastardo Negro and is different from Mollar Cano or Negramoll, which are homonyms of the former one, and a source of frequent mistakes.

Origin and distinctiveness of the cultivated grapevines in Portugal

As mentioned before, there is no overlap between 134 of the obtained genotypes and the genotypes of the SNP-Community Plant Variety Office (CPVO) database. For this reason, it is thought that they probably originated on the Portuguese territory. This finding emphasises the richness of the Portuguese grapevine germplasm. Of the 102 cultivars already present in the ICVV-SNP database, 61 are most probably Portuguese, including eight of the 14 new synonyms identified (Alvarelão Ceitão, Batoca, Caracol, Carrega Branco, Malvasia Babosa, Pintosa, Roal and Verdial), which have historical references in Portugal. Of the last 41 matching cultivars, 15 are also present in Spain: five are important for wine production in Denominação de Origem Controlada and Denominaciones de Origen Calificada (DOC) regions, both in Portugal and Spain, namely, Aragonez/Tempranillo, Jaen/Mencia, Loureiro/Loureiro Blanco, Malvasia Rei/Palomino Fino and Negra Mole/Mollar Cano; nine cultivars have presently a minor presence in Portugal but are, or were, relevant in Spain: Alicante Branco/Planta Fina, Arjunção/Listan Prieto, Corropio/Rayada Melonera, Diagalves/Mantuo, Manteúdo/Listan del Condado, Mourisco/Castañal, Mourisco Branco/Hebén, Sarigo/Cayetana Blanca and Tinta Gorda/Mouraton. Finally, the cultivar Perrum has certainly an Iberian origin, but cannot be specifically assigned to any country, because of the existence of many homonyms and misnames.

A relevant number (23) of matches with non-Iberian cultivars was also found. Seventeen cultivars have a French prime name in the *V*IVC database (Table S1): Amor-Não-Me-Deixes/Aramon Noir, Bastardo/Trousseau Noir, Branco Especial/Madeleine Royale, Espadeiro Mole/Manseng Noir, Mondet/Durif, Pé Comprido/Bourboulenc, Pical/Piquepoul Noir, Rabigato Franco/Grec Rouge, Sevilhão/Corbeau, Tinta Penajoia/Peloursin, Uva Salsa/Chasselas Cioutat, Teinturier/Teinturier, Rodo/Mondeuse Noir, as well as four out of the 14 new synonyms (Malvasia Branca/Gros Vert Blanc, Santareno/Etraire de la Dui, Moscadet/Meslier Saint François, Castelino/Aubun/Corvo). Within this group, only two cultivars (Espadeiro Mole/Manseng Noir and Bastardo/Trousseau Noir) are commonly used for wine production in Portugal. Bastardo/Trousseau Noir is a recommended grape cultivar in the Porto region and has been mentioned in the region since 1532 (Fernandes 1532). Because of its susceptibility to powdery mildew, Espadeiro Mole (Manseng Noir) is not cultivated in the Vinhos Verdes region at least since the end of the 19th century (Menezes 1900).

Two cultivars legally accepted for wine production in Portugal have an Italian prime name in the *V*IVC database: Caramela/Luglienga Bianca and Cornichon/ Cornichon Blanc. These are minor cultivars present only in collections.

The Portugieser Blau/Português Azul is a cultivar that has an Austrian prime name in the *V*IVC database. This cultivar was taken to the Austrian region of Vöslau in 1772 by the Ambassador of Austria in Portugal, and its cultivation extended to several winegrowing regions of Central Europe (Regner et al. 1999). Although a minor cultivar, it is used in breeding programs in Central Europe.

One originally Greek cultivar with the prime name Muscat à Petits Grains Blancs in the *V*IVC database is known in Portugal as Moscatel Galego Branco. It is an old cultivar, with many synonyms (305) and cultivated in many countries.

The tablegrape cultivar Ferral/Ahmeur Bou Ahmeur is originally from Northern Africa and is cultivated both in Portugal and Spain since the Middle Ages (Herrera 1513, Fernandes 1532). Ferral is used in 14 cultivar names in the *V*IVC database, corresponding to ten prime names and is a clear case of multiple homonymies. Its common morphological characteristics are a long and wide berry with an obtuse ovoid shape and red skin colour.

Branco Valente, a synonym of Heunisch Weiss (*V*IVC_5374) (Table S1), is an old cultivar spread all over Europe with 213 synonyms in the *V*IVC database (http://www.vivc.de/). From Heunisch Weiss, 124 first-degree relationships are known (Maul et al. 2015).

Genetic relationships among the Portuguese germplasm

The hierarchical clustering of the 263 unique SNP genotypes using UPGMA produced three clusters (I, II and III), with three exceptions (Figure 2, arrows indicate the outliers): one wild vine genotype from the Grândola population (PRT051-120307) and two cultivars, Folha de Figueira (PRT051-51514) and Aragonez (PRT051-52603).

Clusters I and II include only Portuguese genotypes (cultivated and wild). Cluster I includes all wild genotypes (except PRT051-120307) and two subclusters of grapevine cultivars. One subcluster is formed by one wild vine (PRT051-110603) and 28 cultivars. The second subcluster includes wild grapevines and five cultivars: Barcelo (PRT051-52407), Branjo (PRT051-41202), Melhorio (PRT051-41207), Moscatel Galego

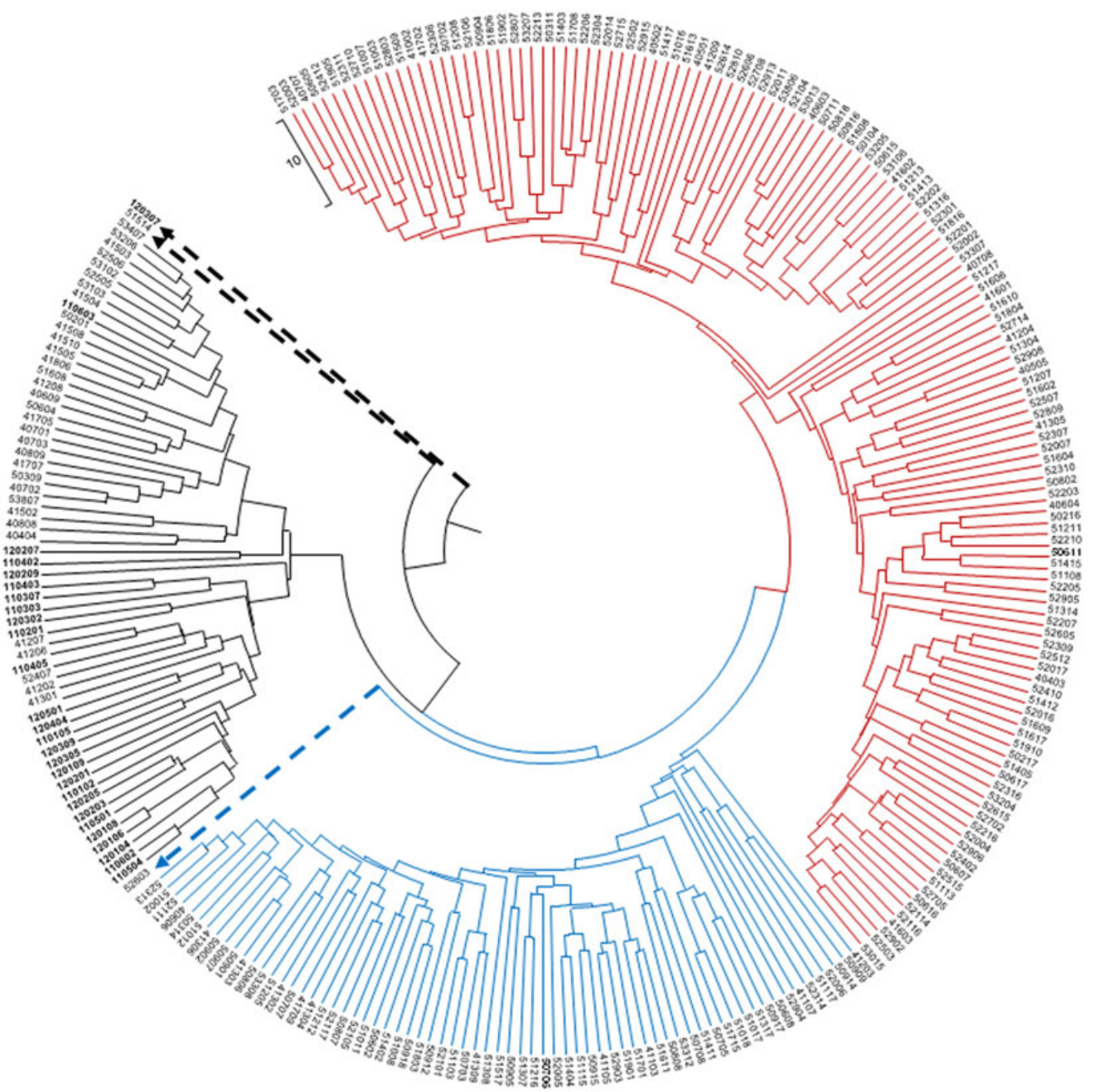

Figure 2. Dendrogram of 263 non-redundant genotypes (grapevine cultivars and wild grapevines) based on the unweighted pair group method with arithmetic mean averages with a squared distances matrix generated with the allelic data from all the 48 single-nucleotide polymorphisms analysed. Cluster I (——); cluster II (——); cluster III (——). Arrows point out the outliers. Wild grapevines are in bold.

Tinto (PRT051-41301) and Transâncora (PRT051-41206). Interestingly, these five cultivars are cultivated mainly in the north of Portugal (Table 1), while all the wild vine genotypes analysed in this work came from southern populations (more than 400 km of linear distance).

Cluster II includes mostly minor Portuguese grapevine cultivars largely from the north of Portugal (84.2%): Douro region (60.3%), Vinhos Verdes region (17.6%) and other northern areas (7.3%) (Figure 1, regions 1, 2, 3 and 4).

Cluster III groups together all the important Portuguese wine cultivars, some of which are major nodes in the genetic network of the Portuguese grapevine germplasm: Amaral, Alfrocheiro, Heben/Mourisco Branco, Cayetana Blanca/Sarigo (Zinelabidine et al. 2012, 2015, Lacombe et al. 2013, Cunha et al. 2015, Maul and Töpfer 2015). Other Iberian and French cultivars are also grouped in this cluster. The proportion of cultivars from Northern Portugal (66%) and from the Lisbon region (21%) present in this cluster reflects the geographical origin of grapevine accessions present in PNAC. In fact, the greatest variability was collected around these two historical winegrowing areas.

A non-hierarchical PCoA based on the squared distances matrix was also used to analyse the relationships among the unique genotypes (Figure 3). The first three axes explain 30.12% of the total variance (13.74, 9.08 and 7.30%, respectively). The plot of Figure 3, basically, confirms the three clusters obtained with the UPGMA clustering (Figure 2).

No agreement was found between the clusters obtained in this work and other cluster analysis obtained using SSR (Lopes et al. 1999, Almadanim et al. 2007, Castro et al. 2011), or morphological descriptions (Cunha 2009) over sets

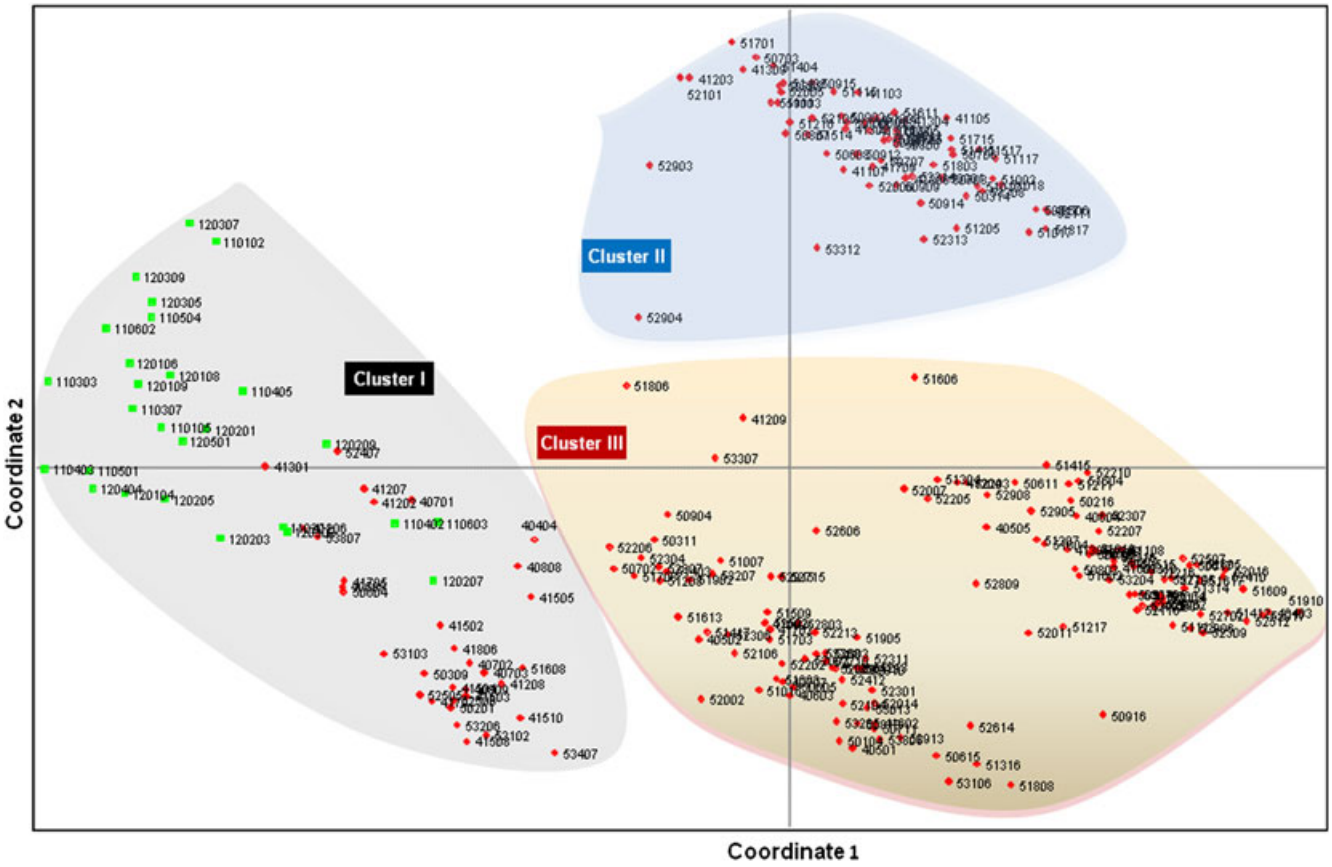

Figure 3. Plot of Portuguese *Vitis vinifera* wild grapevines (■) and grapevine cultivars (◆) diversity from principal coordinate analysis based on a 48-set of single-nucleotide polymorphism markers and via covariance matrix with data standardisation.

of Portuguese accessions. This may be due to a greater number and a better distribution in the genome of the markers used in this work, to the inclusion of a different set of accessions, including various wild grapevines, or to the effect of lower SNP mutation rates when compared with SSR (Cabezas et al. 2011). The four cpSSR profiles (A, B, C and D) previously identified in the Portuguese genotypes (Cunha 2009,Cunha et al. 2009, Castro et al. 2012) were scattered in all three clusters.

Assignment tests for the groups of wild and cultivated grapes showed that 98% of the cultivars and 98% of the wild grapevines are assigned to their own group. Barcelo (PRT051-52407) was the only cultivar assigned to the wild group (as it was in the previous UPGMA and PCoA analysis). Lacerda Lobo (1790) was the first author to refer to Barcelo, a white cultivar presenting the chlorotype D, and a minor cultivar from Lafões (Figure 1, region 4). All the Portuguese wild grapevines bear chlorotypes A or B, and their grapes have a blue-black colour (Cunha et al. 2013). Three wild grapevine genotypes (PRT051-110201, -110603 and -120207) were assigned as cultivars. These accessions are a female, a hermaphrodite and a male plant, respectively, collected in three different populations from three different southern hydrological basins. A previous analysis based on six SSRs identified accession 110603 as a feral plant, but not accession 110201 (Cunha 2009). The male accession 120207 was not previously analysed. It is not uncommon to misidentify feral plants when collecting *sylvestris* samples. These individuals, however, in particular accession 120307, may also be hybrids, because of the ease with which the two subspecies may hybridise (Martínez-Zapater et al. 2013).

Conclusions

Profiling 288 accessions of the Portuguese National Ampelographic collection with a set of 48 SNP markers, 263 non-redundant genotypes were identified, including 27 corresponding to wild grapevines. Fourteen new synonyms were identified among Portuguese, Spanish and other international cultivars. Thirty-three synonyms previously identified using morphological descriptors and/or SSR markers were confirmed. The correct identification of the accessions in the different collections is fundamental for the correct management and preservation of *V. vinifera* germplasm and for the exchange of plant materials.

This analysis showed the existence of a large number of genotypes exclusively present in Portugal or with a residual presence in neighbouring Spain. An important number of cultivars are present in both Iberian countries, and only a small proportion of non-Iberian cultivars, mainly French, is present in Portugal. The Portuguese *V. vinifera* germplasm could be grouped into three clusters, one of which includes all but one of the analysed wild genotypes. The other two clusters contain only cultivated grapevines, one grouping together almost only Portuguese minor cultivars and the other one all the most relevant Portuguese and foreign grapevine cultivars.

The knowledge obtained and the database created will contribute to the management of the Portuguese germplasm and will help adjusting the Portuguese legal framework of the denomination of the cultivars authorised for wine production.

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References

Agüero, C.B., Rodriguez, J.G., Martinez, L.E., Dangl, G.S. and Meredith, C.P. (2003) Identity and parentage of Torrontes cultivars in Argentina. American Journal of Enology and Viticulture **54**, 318–321.

Alarte, V. (1712) Agricultura das vinhas, e tudo o que pertence a ellas, até perfeito recolhimento do vinho, e relação das suas virtudes, e da cêpa, vides, folhas e borras (Officina Real Deslandesiana: Lisbon, Portugal).

Almadanim, M.C., Baleiras-Couto, M.M., Pereira, H.S., Carneiro, L.C., Fevereiro, P., Eiras-Dias, J.E., Morais-Cecilio, L., Viegas, W. and Veloso, M.M. (2007) Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal. Vitis **46**, 116–119.

Arroyo-García, R., Lefort, F., de Andrés, M., Ibáñez, J., Borrego, J., Jouve, N., Cabello, F. and Martínez-Zapater, J.M. (2002) Chloroplast microsatellite polymorphisms in *Vitis* species. Genome **45**, 1142–1149.

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

Borrego, J., de Andres, M.T., Gomez, J.L. and Ibáñez, J. (2002) Genetic study of Malvasia and Torrontes groups through molecular markers. American Journal of Enology and Viticulture **53**, 125–130.

Bowers, J.E. and Meredith, C.P. (1996) The parentage of classic wine grape: Cabernet Sauvignon. Nature Genetics **16**, 84–87.

Cabello, F., Ortz, J.M., Muñoz, G., Rodríguez-Torres, I., Benito, A., Rubio, C., García-Muñoz, S. and Sáiz, R. (2011) Variedades de vid en España (Editorial Agrícola Española: Madrid, España).

Cabezas, J.A., Ibáñez, J., Lijavetzky, D., Vélez, D., Bravo, G., Rodríguez, V., Carreño, I., Jermakow, A.M., Carreño, J., Ruiz-Garcia, L., Thomas, M.R. and Martínez-Zapater, J.M. (2011) A 48 SNP set for grapevine cultivar identification. BMC Plant Biology **11**, 153.

Castro, I., Martín, J.P., Ortiz, J.M. and Pinto-Carnide, O. (2011) Varietal discrimination and genetic relationships of *Vitis vinifera* L. cultivars from two major Controlled Appellation (DOC) regions in Portugal. Scientia Horticulturae **127**, 507–514.

Castro, I., Martin, J.P., Ortiz, J.M., Mota, M.T. and Pinto-Carnide, O. (2012) The Portuguese grapevine cultivar 'Amaral': synonymies, homonymies and misnames. Vitis **51**, 61–63.

Cunha, J. (2009) Biological diversity of *Vitis vinifera* L. in Portugal: the genetic contribution of subsp. *sylvestris* to the origin of the Portuguese grapevine cultivars (subsp. *vinifera*). PhD thesis. Instituto de Tecnologia Química e Biológia (ITQB), Universidade Nova de Lisboa, Lisbon, Portugal.

Cunha, J., Teixeira-Santos, M., Brazão, J., Fevereiro, P. and Eiras-Dias, J.E. (2013) Portuguese *Vitis vinifera* L. germplasm: accessing its diversity and strategies for conservation. Poljuha, D. and Sladonja, B., eds. The Mediterranean genetic code—grapevine and olive (InTech: Rijeka, Croatia) pp. 125–144.

Cunha, J., Teixeira Santos, M., Carneiro, L., Fevereiro, P. and Eiras-Dias, J. (2009) Portuguese traditional grapevine cultivars and wild vines (*Vitis vinifera* L.) share morphological and genetic traits. Genetic Resources and Crop Evolution **56**, 975–989.

Cunha, J., Teixeira-Santos, M., Veloso, M., Carneiro, L., Eiras-Dias, J. and Fevereiro, P. (2010) The Portuguese *Vitis vinifera* L. germplasm: genetic relations between wild and cultivated vines. Ciência e Técnica Vitivinícola **25**, 25–37.

Cunha, J., Zinelabidine, L.H., Teixeira-Santos, M., Brazão, J., Fevereiro, P., Martinez-Zapater, J.M., Ibáñez, J. and Eiras-Dias, J. E. (2015) Grapevine cultivar "Alfrocheiro" or "Bastardo Negro" plays a primary role in Iberian grapevine diversity. Vitis(Special issue) **54**, 59–65.

de Andrés, M.T., Benito, A., Pérez-Rivera, G., Ocete, R., Lopez, M.A., Gaforio, L., Muñoz, G., Cabello, F., Martínez-Zapater, J.M. and Arroyo-García, R. (2012) Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. Molecular Ecology **21**, 800–816.

Dong, Q.-H., Cao, X., Yang, G., Yu, H.-P., Nicholas, K.K., Wang, C. and Fang, J.-G. (2010) Discovery and characterization of SNPs in *Vitis vinifera* and genetic assessment of some grapevine cultivars. Scientia Horticulturae **125**, 233–238.

Eiras-Dias, J.E., Faustino, R., Clímaco, P., Fernandes, P., Cruz, A., Cunha, J., Veloso, M. and Castro, R. (2011) Catálogo das castas para vinho cultivadas em Portugal. Volume 1. Instituto da Vinha e do Vinho I.P. (Chaves Ferreira—Publicações: Lisbon, Portugal).

Eiras-Dias, J.E., Faustino, R., Clímaco, P., Fernandes, P., Cruz, A., Cunha, J., Veloso, M. and Castro, R. (2013) Catálogo das castas para vinho cultivadas em Portugal. Volume 2. Instituto da Vinha e do Vinho I.P. (Chaves Ferreira—Publicações: Lisbon, Portugal).

Fernandes, R. (1532) Descrição do terreno ao redor de Lamego duas léguas: 1531-1532. J. Barros, A.J.M., ed. Caleidoscópio—Edição e Artes Gráficas 2012 (Casal de Cambra: Portugal).

Fernández-González, M., Mena, A., Izquierdo, P. and Martinez, J. (2007) Genetic characterization of grapevine (*Vitis vinifera* L.) cultivars from Castilla La Mancha (Spain) using microsatellite markers. Vitis **46**, 126–130.

Gago, P., Santiago, J.L., Boso, S., Alonso-Villaverde, V., Grando, M.S. and Martínez, M.C. (2009) Biodiversity and characterization of twenty-two *Vitis vinifera* L. cultivars in the northwestern Iberian Peninsula. American Journal of Enology and Viticulture **60**, 293–301.

Herrera, A.D. 1513. Agricultura general. Edición facsimil (1981) (Servicio de Publicaciones del Ministerio 268 de Agricultura y Pesca: Madrid, Spain).

Ibáñez, J., de Andrés, M.T., Molino, A. and Borrego, J. (2003) Genetic study of key Spanish grapevine varieties using microsatellite analysis. American Journal of Enology and Viticulture **54**, 22–30.

Ibáñez, J., Muñoz-Organero, G., Zinelabidine, L.H., de Andrés, M.T., Cabello, F. and Martíñez-Zapater, J.M. (2012) Genetic origin of the grapevine cultivar Tempranillo. American Journal of Enology and Viticulture **63**, 549–553.

Instituto Nacional de Estatística (2014) Estatísticas agrícolas 2013. Instituto Nacional de Estatística website. https://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=210756829&PUBLICACOESmodo=2 [accessed February 2015].

Instituto da Vinha e do Vinho (1991) Cadastro vitícola: Curso de ampelografia e sinonímia das variedades de videira realizado no complexo tecnológico do Catujal. Trabalho realizado no âmbito das acções de pré-adesão Portugal-Comunidade Económica Europeia. Relatório e conclusões (Instituto da Vinha e do Vinho: Lisbon, Portugal).

Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R. and Pandey, B.K. (2009) Potential of molecular markers in plant biotechnology. Plant Omics Journal **2**, 141–162.

Lacerda Lobo, C. (1790) Memoria sobre a cultura das vinhas em Portugal (Academia Real das Sciencias de Lisboa: Lisbon, Portugal).

Lacombe, T., Boursiquot, J.-M., Laucou, V., Vecchi-Staraz, M., Péros, J.-P. and This, P. (2013) Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.) Theoretical and Applied Genetics **126**, 401–414.

Lijavetzky, D., Cabezas, J., Ibanez, A., Rodriguez, V. and Martinez-Zapater, J. (2007) High throughput SNP discovery and genotyping in grapevine (*Vitis vinifera* L.) by combining a re-sequencing approach and SNPlex technology. BMC Plant Biology **8**, 424.

Lopes, M.S., Rodrigues dos Santos, M., Eiras-Dias, J.E., Mendonça, D. and Câmara Machado, A. (2006) Discrimination of Portuguese grapevines based on microsatellite markers. Journal of Biotechnology **127**, 34–44.

Lopes, M.S., Sefc, K.M., Eiras-Dias, E., Steinkellner, H., Laimer Câmara Machado, M. and Câmara Machado, A. (1999) The use of microsatellites for germplasm management in a Portuguese grapevine collection. Theoretical and Applied Genetics **99**, 733–739.

MAMAOT (2012) Portaria n° 380/2012, de 22 de Novembro, do Ministério da Agricultura, do Mar, do Ambiente e do Ordenamento

do Território (MAMAOT). Diário da República, 1.ª série - N.° 226. Lisbon, Portugal.
Martín, J.P., Borrego, J., Cabello, F. and Ortiz, J.M. (2003) Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers. Genome **46**, 10–18.
Martín, J.P., Santiago, J.L., Pinto-Carnide, O., Leal, F., Martinez, M.D. and Ortiz, J.M. (2006) Determination of relationships among autochthonous grapevine varieties (*Vitis vinifera* L.) in the Northwest of the Iberian Peninsula by using microsatellite markers. Genetic Resources and Crop Evolution **53**, 1255–1261.
Martínez-Zapater, J.M., Lijavetzky, D., Fernández, L., Santana, J.C. and Ibáñez, J. (2013) The history written in the grapevine genome. Pérez, S.C. and Pérez, J.B., eds. Vine and wine cultural Heritage (UAM Ediciones: Madrid, Spain) pp. 213–231.
Maul, E., Eibach, R., Zyprian, E. and Töpfer, R. (2015) The profilic grape variety (*Vitis vinivera* L.) 'Heunisch Weiss' (='Gouais blanc'): bud mutants, "colored" homonyms and further offspring. Vitis **54**, 79–86.
Maul, E. and Töpfer, R. (2015) Vitis International Variety Catalogue (*VI*VC): a cultivar database referenced by genetic profiles and morphology. BIO Web of Conferences 5, 01009.
Menezes, P. (1896) Apontamentos para o estudo da ampelographia Portugueza. Boletim da Direcção Geral de Agricultura (Ministério das Obras Publicas, Comercio e Industria, Imprensa Nacional: Lisbon, Portugal).
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: Lisbon, Portugal).
Morin, P.A., Luikart, G., Wayne, R.K. and the SNP workshop group (2004) SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution **19**, 208–216.
Myles, S., Boyko, A.R., Owens, C.L., Brown, P.J., Grassi, F., Aradhya, M.K., Prins, B., Reynolds, A., Chia, J.-M., Ware, D., Bustamante, C. D. and Buckler, E.S. (2011) Genetic structure and domestication history of the grape. Proceedings of the National Academy of Sciences of the United States of America **108**, 3530–3535.
Organisation Internationale de la Vigne et du Vin (1983) Descriptor list for grapevine varieties and *Vitis* species (Organisation Internationale de la Vigne et du Vin: Paris, France).
Organisation Internationale de la Vigne et du Vin (2009) Descriptor list for grapevine varieties and *Vitis* species, 2nd edn (Organisation Internationale de la Vigne et du Vin: Paris, France).
Organisation Internationale de la Vigne et du Vin (2013) Statistical report on world vitiviniculture. Office Internationale de la Vigne et du Vin website. http://www.oiv.int/oiv/info/enstatsro [accessed February 2015].
Park, S.D.E. (2001) The excel Microsatellite Toolkit v.3.1. Animal Genomics Laboratory (University College Dublin : Dublin, Ireland).
Peakall, R. and Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics **28**, 2537–2539.
Pl@ntGrape (2007). Catalogue of vines grown in France. Pl@ntGrape website. http://plantgrape.plantnet-project.org/cepage/Etraire%20de%20la%20Dui%20N [accessed February 2015].
Regner, F., Eiras-Dias, J.E., Stadbauer, A. and Blahous, D. (1999) 'Blauer Portugieser', the dissemination of a grapevine. Ciência e Técnica Vitivinícola **14**, 37–44.
Reis, J.C.D.C. (1986) Projecto de Cadastro viticola (Instituto de Gestão e Estrutura Fundiária e Instituto da Vinha e do Vinho, Ministério da Agricultura Florestas e Alimentação: Lisbon, Portugal).
Rodríguez-Torres, I. (2013) Descriptores para la caracterización de vid. Variedades cultivadas en Canarias (Instituto Canario de Investigaciones Agrarias: Gobierno de Canarias: Spain).
Sefc, K.M., Lopes, M.S., Lefort, F., Botta, R., Roubelakis-Angelakis, K.A., Ibáñez, J., Pejic, I., Wagner, H.W., Glossl, J. and Steinkellner, H. (2000) Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theoretical and Applied Genetics **100**, 498–505.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution **28**, 2731–2739.
Thomas, M.R., Matsumoto, S., Cain, P. and Scott, N.S. (1993) Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theoretical and Applied Genetics **86**, 173–180.
Veloso, M.M., Almandanim, M.C., Baleiras-Couto, M., Pereira, H.S., Carneiro, L.C., Fevereiro, P. and Eiras-Dias, J. (2010) Microsatellite database of grapevine (*Vitis vinifera* L.) cultivars used for wine production in Portugal. Ciência e Técnica Vitivinícola **25**, 53–61.
Viala, P. and Vermorel, V. (1905-1910) Traité général de viticulture, ampélographie. Vol. 1–7 (bib. code 1). (Masson et Compagnie: Paris, France).
Vilanova, M., de la Fuente, M., Fernández-González, M. and Masa, A. (2009) Identification of new synonymies in minority grapevine cultivars from Galicia (Spain) using microsatellite analysis. American Journal of Enology and Viticulture **60**, 236–240.
Villa Maior, V.D. (1866) Preliminares da ampelografia e enologia do país vinhateiro do Alto Douro. 2° e 3° fasc. (Imprensa Nacional: Lisbon, Portugal).
Zinelabidine, L.H., Cunha, J., Eiras-Dias, J.E., Cabello, F., Martínez-Zapater, J.M. and Ibáñez, J. (2015) Pedigree analysis of the Spanish grapevine cultivar Heben. Vitis(special issue) **54**, 81–86.
Zinelabidine, L.H., Haddioui, A., Rodriguez, V., Cabello, F., Eiras-Dias, J. E., Martinez-Zapater, J.M. and Ibáñez, J. (2012) Identification by SNP analysis of a major role for Cayetana Blanca in the genetic network of Iberian Peninsula grapevine varieties. American Journal of Enology and Viticulture **63**, 121–126.
Zinelabidine, L.H., Laiadi, Z., Benmehaia, R., Gago, P., Boso, S., Santiago, J.L., Haddioui, A., Ibáñez, J., Martínez-Zapater, J.M. and Martínez, M.C. (2014) Comparative ampelographic and genetic analysis of grapevine cultivars from Algeria and Morocco. Australian Journal of Grape and Wine Research **20**, 324–333.

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Supporting information

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Table S1. Single-nucleotide polymorphism genotypes for the 263 non redundant Portuguese autochthonous cultivars.