Chapter 6

Portuguese *Vitis vinifera* L. Germplasm: Accessing Its Diversity and Strategies for Conservation

Jorge Cunha, Margarida Teixeira-Santos,
João Brazão, Pedro Fevereiro and
José Eduardo Eiras-Dias

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1. Introduction

1.1. Economical, cultural and historical importance of grapevine in Portugal

Grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world. In the different Portuguese agro-ecosystems, grapevine plays an important role either as a border culture or as an extensive crop. The surface area used by vineyards amounts to 4.9 % of the arable land [1], representing 240,000 ha, being the 7th largest area in the world and the 4th in the European Union [2]. In 2011 Portugal produced 5.9 million hectoliters of which 2.9 million hectoliters were exported, making the country the 12th world wine producer [2]. There are fourteen wine regions with Protected Geographical Indication (Figure 1) and 31 wine areas with Designation of Origin status including Porto, established since 1756, the oldest legally established wine production region in the world. Each one of the wine regions has a particular set of grapevine cultivars adapted to its specific *terroirs*. Officially there are 343 cultivars allowed to be use in wine production in Portugal [3].

Grapes were eaten by Neolithic and Bronze Age populations of the Iberian Peninsula since the 3rd millennium BCE as proven by archaeological remains [4, 5, 6]. Consumption and production of wine is thought to have started by the Iberian populations in contact with the Phoenicians and Greeks trading ports. It further expanded during the Roman occupation and reach important religious prominence with the Christianization of population. It even continued during the Muslim caliphate since part of the population maintain the Christian faith. After the 10th century convents and monasteries spread again grapevine cultivation and implemented new tools for wine production. Since the 12th century, Portugal produces wine not only for local consumption but also for export, especially to northern Europe. This remote history of grapevine cultivation allowed the building up of great diversity. The number of cultivars increased until the tree waves of destruction from North American pest and diseases: powdery mildew (*Uncinula necator* Schweinf. Burrill) in 1851, phylloxera (*Dactylosphaera vitifoliae* Fitch) in 1863 and downy mildew [*Plasmopara viticola* (Berk. & M.A. Curtis) Berl & de Toni] in 1880. Until these severe pathological events grapevine was multiply simply by self-rooting of cutting our seed germination. Since the introduction of phylloxera the use of rootstocks from hybrids of other *Vitis* species is mandatory, except in areas were the phylloxera cannot survive. Such a case occurs in the Designation of Origin Colares wine region where the vineyards are settled in sandy soil and the roots are over tree meters deep. As early as the 19th century attempts to improve grape production result in a number of cultivars as Tinta do Aurélio (red cultivar selected by someone called "Aurélio"). However a truth breeding program to obtain new varieties was only started in the mid of the 20th century by José Leão Ferreira de Almeida and two of the obtain cultivars, Dona Maria (table grape) and Seara Nova (wine grape), occupy today a significant acreage [7]. The exact number of cultivars in use is unknown but from the 340 allowed for wine production, 240 are thought to be autochthonous [8, 9].

Figure 1. Location of the Portuguese wine regions. (Source: Wines of Portugal - http://www.winesofportugal.info/pagina.php?codNode=18012).

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Traditionally morphological descriptors were used to characterize cultivars until the advent of molecular markers. Presently these have been successfully used in a wide range of applications such as assessing genetic diversity [10], linkage mapping [11], cultivar identification and pedigree studies [12], [13]. Microsatellites (SSR) are being used to characterize grapevine cultivars and wild vines [10, 14] and to carry out genetic diversity analyses [15]. Usually six *loci* are sufficient for differentiating between genotypes [16], but closely related cultivars require a larger number of *loci* [17]. Sequence variation at the chloroplastidial *loci* has been extensively used to assess phylogenetic relationships among plant *taxa*, based on their low rate of sequence evolution, the almost absent recombination and single parent inheritance [18]. All this range of tools is useful to make decisions on the strategies for conservation.

2. Diversity of the Portuguese grape germplasm

2.1. Wild vine populations: Geographical distribution, morphological and molecular characterization

Wild vine populations of *Vitis vinifera* L. subspecies *sylvestris* [(Gmelin) Hegi)] is closely related to the cultivated grapevine (*Vitis vinifera* subsp. *vinifera*), first domesticated 10,000 years BP around the Caspian Sea [19]. In Portugal these wild vine populations are distributed along riparian woods and flooded river banks in the southern part of the country in what is the most western habitats of this subspecies. From the Atlantic coasts of southwest Europe and northwest Africa this subspecies is distributed in patches adjacent to rivers along the Mediterranean basin, Central Europe and in Asia between the Black Sea and the Hindu Kush [20]. Once this subspecies occupied a larger area as a result of the its expansion after the last Quaternary glaciations [21, 22] but today´s remaining areas are refuges from human pressure and North-American pest and diseases introduced during the 19th century. Human populations since the early settlements in the Iberian Peninsula collected and consumed wild grapes [6] and this resource continued to be used until the late 20th century in folk medicine [20].

The wild vine populations found up to now in Portugal live in riparian woods along small streams (Figure 2) belonging to three large river basins – Tagus (Tejo in Portuguese), Guadiana and Sado (Table 1). The first two rivers are common to Portugal and Spain and the populations along these basins, even if found in patches, could be considered as a continuum [23, 24].

In these riparian woods the plants species most frequently found as tutors of V*itis vinifera* L. ssp. *sylvestris* are: *Adenocarpus complicatus*, *Alnus glutinosa*, *Fraxinus angustifolia*, *Nerium oleander*, *Olea europea*, *Quercus faginea* subsp. *Broteroi*, *Quercus suber*, *Rubus ulmifolius*, *Salix atrocinerea*, *Salix neotricha* and *Salix salvifolia* subsp. *salvifolia* [23, 25] . The thirteen populations found until now (Table 1) thrive in a typically Mediterranean environment. Fifty three plants belonging to four of these populations were characterized morphologically using the OIV [26] and GENRES-081 [27] descriptors [23, 28, 29].

Figure 2. *Vitis vinifera* subspecies *sylvestris* male plant from the São José/ Toutalga population in its natural habitat, a riparian forest along a small stream from the Guadiana river basin.

Population	River basin	Reference Code	Latitude	Longitude	Elevation (meters)	Estimated size of the population	PopRisk
St[a] Sofia - Montemor-o-Novo	Tagus	01*[a]	38°36'41''N	08°05'24''W	306	[30-40]	3
Pônsul - Castelo Branco	Tagus	02*[a]	39°45'16''N	07°26'06''W	119	[30-40]	7
Guadiana - Mourão	Guadiana	03[a]	38°24'10''N	07°22'36''W	128	0	9
Vale do Guiso - Alcácer do Sal	Sado	04*[a]	38°14'46''N	08°22'30''W	49	[10-20]	3
Portel	Guadiana	05*	38°16'46''N	07°38'07''W	197	[20-30]	7
Ardila - Barrancos	Guadiana	06	38°07'56''N	06°57'41''W	208	[20-30]	5
Vendinha - Évora	Guadiana	07	38°27'18''N	07°41'02''W	163	[10-20]	5
Pintada - Montemor-o-Novo	Tagus	08	38°37'59''N	08°11'31''W	204	[10-20]	5
Fronteira	Tagus	09	39°02'38''N	07°42'14''W	93	[10-20]	5
Anta do Silval - Évora	Tagus	10	38°36'45''N	08°03'29''W	292	<10	5
Q. do Pinheiro - Montemor-o-Novo	Tagus	11	38°37'58''N	08°10'31''W	234	[20-30]	5
S.José/Toutalga - Moura	Guadiana	12	38°02'37''N	07°15'54''W	176	[20-30]	5
Enxota tordos - Grândola	Sado	13	38°13'27''N	08°30'22''W	34	>50	3

* Wild populations studied by [28, 29]/ [a] Wild populations studied by [33]

PopRisk (survival risk of the population): 1= No Risk; 3= Some Risk; 5= Medium Risk; 7= At Risk; 9= Extinct

Table 1. *Vitis vinifera* ssp. *sylvestris* Portuguese populations data: River basin; geographic coordinates, elevation (in meters) estimated size of the population, and risk of extinction.

The characterized wild vine plants featured the particularly morphological characteristics of the subspecies *sylvestris*: i) open young shoots, which is a characteristic allowing to differentiate between *Vitis vinifera* and the other *Vitis* species and hybrids; ii) the presence of male and female plants in each population (dioecious plants) (hermaphrodite plants are rare in wild vine populations and the rule in cultivated grapevines); iii) Stummer's Index (breadth/length ratio x 100) [30] of pips is equal or greater than 75 in wild vines. The morphological characteristics of the leaves, shoots and bunches were used to distinguish different phenotypes in the field. Until now only blue black berries were found and the ratio of male to female plants varies from population to population [28]. The 53 different wild vine accessions collected were genotyped using the six nuclear microsatellites suggested by the OIV [31, 32]. The diversity founded in wild vine genotypes (Table 2) reveals that the observed Heterozigocity (Ho) was less than the expected Heterozigocity (He) in all *loci*, confirming the result obtain in a different group of accessions from the same populations using a set of 11 SSRs [33] .

Locus	N	Na	Ne	Ho	He	F
VVMD5	53	10	2.428	0.585	0.588	0.005
VVMD7	53	9	2.869	0.547	0.651	0.160
VVMD27	53	8	3.417	0.509	0.707	0.280
VRZag 62	53	7	2.917	0.585	0.657	0.110
VRZag79	53	8	2.884	0.642	0.653	0.018
VVS2	53	11	5.021	0.736	0.801	0.081

Table 2. Diversity obtained in 53 Portuguese wild vines: *locus*, accessions number (*N*), number of alleles (*Na*), number of effective alleles (*Ne*), observed Heterozygosity (Ho), expected Heterozygosity (*He*) and Fixation Index (*F*).

The values of the Fixation Index (F) range from 0.005 to 0.28, showing the existence of inbreeding in some wild vine populations, since F is expected to be close to zero under random mating [34].

An Analysis of Molecular Variance (AMOVA) performed on the same molecular data showed that the genetic diversity was attributable to differences among individuals within populations (93.0%), but Fst values among populations are still significant (*Fst* = 0.071; P, 0.001), showing a low inter-population differentiation (Table 3). The morphological and molecular data confirmed that some of the collected plants were clones due to vegetative propagation (asexual propagation), but that the majority were different genotypes arising from seeds (sexual propagation).

Chloroplastidial microsatellites (cpSSRs) have been used to study the genetic relationships among grapevine cultivars [35], wild vines [36] and relations between both subspecies [37, 38]. Analysis of chloropastidial microsatellites (Figure 3) revealed the expected situation for the Iberian Peninsula [37] with the presence of chlorotypes A and B, being chlorotype A the most frequent within the wild vine populations (66%) of Portugal.

Variance component	Degrees of freedom	Sum of Squares	Variance components	Percentage of variation
Among Populations	3	17.0	0.15	7%
Within Populations	102	198.1	1.94	93%
Total	105	215.1	2.09	
Fixation index (Fst)		0.071 (P<0.001)		

Table 3. AMOVA analyses of six nuclear microsatellites data of 53 Portuguese wild vines on four distinct Southern Portuguese populations.

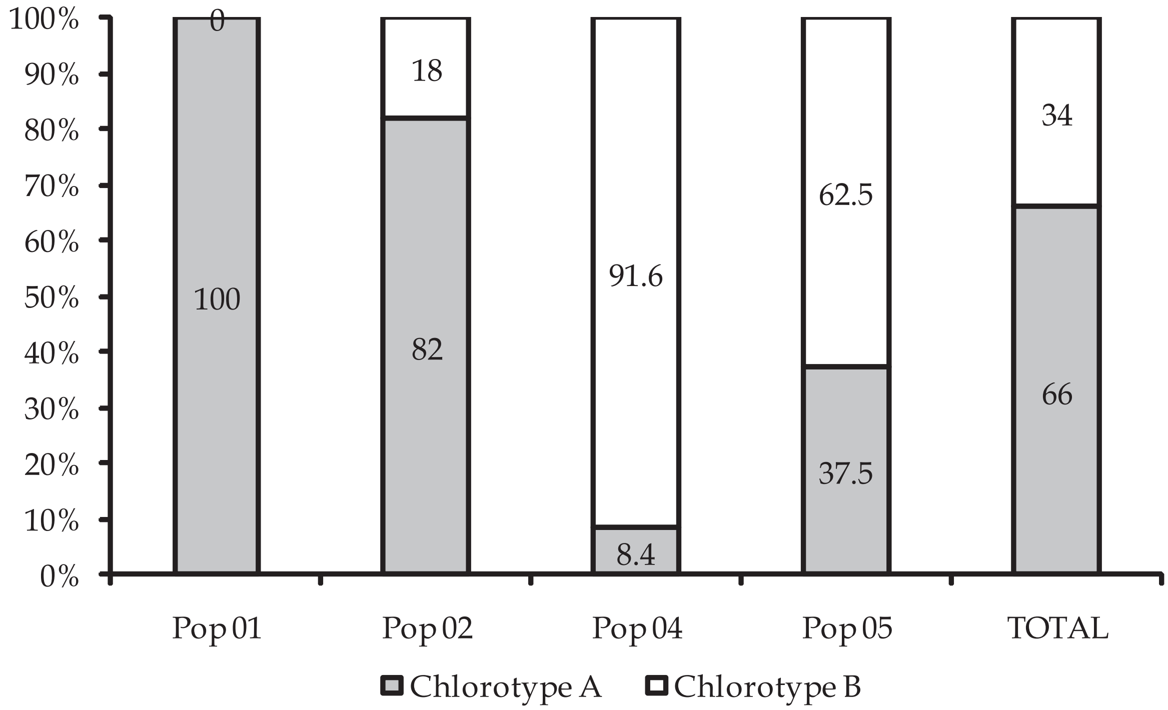

Figure 3. Chlorotypes identified in each Portuguese wild vine population. Chlorotype nomination according to [37].

Chlorotype A is the most frequent in Western Europe and absent in Near East where the domestication of *Vitis vinifera* occurred. The distribution of chlorotypes in four Southern Portuguese populations is heterogeneous. Only chlorotype A was found in plants of the population of Sta Sofia – Montemor-o-Novo. In the populations of Vale do Guiso - Alcácer do Sal, Pônsul – Castelo Branco and Portel both A and B chlorotypes were found but with distributions of 91.6%, 18% and 62.5% of chlorotype B respectively (Figure 3).

2.2. Cultivated grapevine: Morphological and molecular diversity

Portugal, a small country on the outer edge of Europe, has nonetheless a very rich diversity of grapevine cultivars build up over the centuries and back to the 19th century, 1482 different cultivar names were known. To organize the disarray that the different names caused to the wine sector the Ministry of Agriculture promoted a program to sort out the synonyms and homonyms using morphological descriptions [39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49]. Before Portugal joined the EEC (European Economic Community) in 1986, the Ministry of Agriculture finally drew up a list of "authorized" and "recommended" grapevine cultivars for each and every wine production areas (Figure 1). These efforts lead to the establishment of the Portuguese National Ampelografic Collection (in Portuguese "Coleção Ampelográfica Nacional" – CAN; international code PRT051) in 1988 after an extensive survey and collection of accessions all over the country. All CAN accessions were grafted into SO_4 rootstock and each access is represented by seven plants from the same original mother plant. This collection holds 691 accessions of *Vitis vinifera* ssp. *vinifera*; 30 accessions of *Vitis vinifera* ssp. *sylvestris*; 24 accessions of rootstocks and nine of other *Vitis* species. The sanitary status of the collection was also assessed for the principal viruses of grapevine (*Arabis* mosaic virus (ArMV), grapevine fanleaf virus (GFLV), grapevine fleck virus (GFKV), grapevine leafroll associated viruses 1, 2, 3 and 7 (GLRaV 1, 2, 3 and 7) grapevine virus A (GVA) and grapevine virus B (GVB) [50].

The molecular characterization of the Portuguese grapevine cultivars was initiated in 1999 by Lopes and collaborators and a number of known synonyms and homonyms as well as pedigrees were confirmed [51, 52, 53]. A systematic characterization of all the 340 varieties admitted for wine production in Portugal, including 243 autochthonous grape cultivars (Table 4) was done with the six nuclear SSRs recommended by OIV [8, 9]. These studies come to prove the synonyms and homonyms that previous morphologic description had established in the past and also allowed the finding out of new ones.

The diversity present in the 243 autochthonous grapevine cultivars analyzed based on the six nuclear SSRs genetic markers (Table 5) reveals that the observed Heterozigocity (*Ho*) was slightly higher than the expected Heterozigocity (*He*) in all *loci*. The Fixation Index (*F*) is negative for all *loci*, indicating an excess of Heterozigocity, probably due to the strong barrier caused by the vegetative propagation commonly used in grapevine.

Four chlorotypes (A, B, C and D) were found in the autochthonous grapevine cultivars so far genotyped (roughly one quarter of the 243) (Figure 4). Chlorotype A is the most frequent, and it is present in 75% of the cultivars, followed by chlorotype D with 19%. Chlorotypes B and C are each present in a very restricted number of cultivars [29, 32, 37]. These results support the presumption that most of the Portuguese cultivated grapevine germplasm may have derived from local domestication, but that some are the result of introgressed with foreign material as exemplified by important wine cultivars like Touriga Franca and Trincadeira that show the presence of the D chlorotype.

Access number	Grape cultivar	Origin	Access number	Grape cultivar	Origin
40403	Seara Nova	E.A.N.	41703	Malvasia Preta Roxa	Douro.
40404	Assaraky	E.A.N.	41705	Roxo de Vila Flor R	Douro.
40501	Promissão	Douro.	41702	Gouveio Roxo	Douro.
40502	Branco Valente B	Douro.	41707	Deliciosa	E.A.N.
40505	Sercial	Madeira.	41708	Bastardo Roxo	Douro.
40603	Malvasia Babosa B	Madeira.	41709	Donzelinho Roxo	Douro.
40604	Malvasia São Jorge	Madeira.	41806	Campanário	E.A.N.
40606	Granho	Alentejo.	50104	Ferral	unknown
40609	Tinta Aurélio	Douro.	50201	Complexa	E.A.N.
40701	Alvarinho Lilaz B	E.A.N.	50216	Terrantez do Pico	Pico - Açores.
40702	Castália	E.A.N.	50218	Arintaçor	Terceira - Açores.
40703	Naia	E.A.N.	50309	Castelo Branco	E.A.N.
40704	Malvasia de Oeiras B	E.A.N.	50314	Branca de Anadia	E.A.N.
40708	Cornichon	Alentejo.	50317	Verdelho	Açores.
40808	Generosa	E.A.N.	50602	Tinta Martins	Douro.
40809	Rio Grande	E.A.N.	50604	Tinta Mesquita	Douro.
41002	Pé Comprido	Douro.	50605	Português Azul	Douro.
41103	Esganinho	Vinhos Verdes.	50607	Tinta Gorda N	Douro.
41105	Branco Gouvães	Douro.	50608	Tinta Malandra N	Douro.
41107	Branco Desconhecido	Douro.	50611	Lameiro	Vinhos Verdes.
41202	Branjo	Vinhos Verdes.	50615	Água Santa	E.A.N.
41203	Galego	Vinhos Verdes.	50616	Gouveio Real	Douro.
41204	Labrusco	Vinhos Verdes.	50617	Gouveio Estimado	Douro.
41205	Melhorio	Vinhos Verdes.	50702	Mondet	Douro.
41206	Transâncora	Vinhos Verdes.	50703	Tinta Aguiar	Douro.
41208	Verdial Tinto	Douro.	50705	Touriga Fêmea	Douro.
41209	Alvarelhão Ceitão	Douro.	50706	Tinta Miúda de Fontes N	Douro.
41301	Moscatel Galego Tinto	Douro.	50707	Tinta Roseira N	Douro.
41302	Barreto de Semente T	Douro.	50708	Lourela	Douro.
41303	Casteloa	Douro.	50802	Gonçalo Pires	Douro.
41304	Farinheira	Douro.	50806	Padeiro de Basto N	Vinhos Verdes.
41305	Gouveio Preto	Douro.	50807	Tinta Pomar	Douro.
41306	Mourisco de Trevões	Douro.	50808	Tinta Varejoa N	Douro.
41309	Tinta Melra T	Douro.	50901	Casculho	Douro.
41502	Alentejana N	E.A.N.	50902	Concieira	Douro.
41503	Lusitano	E.A.N.	50904	Doçal	Vinhos Verdes.
41504	Tinta de Alcobaça N	E.A.N.	50905	Doçal de Refoios N	Douro.
41505	Agronómica	E.A.N.	50907	Tinta Pereira	Douro.
41508	Portalegre N	E.A.N.	50909	Malvasia Trigueira R	Douro.
41509	Triunfo	E.A.N.	50912	Malvasia Branca	Açores.
41601	Monvedro de Sines N	Sines	50914	Caracol	Madeira.
41603	Manteúdo Preto	Alentejo.	50915	Esganoso	Vinhos Verdes.
41605	Listrão	Madeira.	50916	Mourisco Branco	Douro.
41607	Mindelo	E.A.N.	50917	Rabigato Moreno	Douro.

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50918	Roxo Rei	Douro.
51002	Castelã	Douro.
51003	Amor-não-me-deixes	Alentejo.
51007	Pical-Polho N	Vinhos Verdes.
51008	Tinta Engomada N	Douro.
51011	Sercialinho	E.A.N.
51012	Trincadeira Branca	Estremadura.
51016	Caramela	Douro.
51017	Estreito Macio	Douro.
51018	Branco Guimarães	Douro.
51103	Tinta Ricoca N	Douro.
51108	Bastardo Espanhol N	Beira Interior.
51113	Larião	Alentejo.
51115	Luzidio	Dão.
51117	Bastardo Branco	Douro.
51202	Tinta Negra	Madeira.
51205	Tintinha	Alentejo.
51207	Corvo	Estremadura.
51208	Tinta Roriz de Penajóia N	Douro.
51209	Dedo de Dama	Estremadura.
51211	Uva Cavaco	Beira Interior.
51212	Malvasia Cabral	Douro.
51216	Branco Especial	Douro.
51217	Pintosa	Vinhos Verdes.
51304	Coração de Galo	Dão.
51307	Tinta Tabuaço	Douro.
51308	Tinta de Cidadelhe N	Douro.
51314	Roupeiro B	Estremadura.
51316	Sarigo	Douro.
51317	Côdega de Larinho	Douro.
51402	Mourisco de Semente	Douro.
51403	Sevilhão	Douro.
51404	Cidreiro	Dão.
51405	Corropio	Alentejo.
51410	Douradinha B	Dão.
51411	Dorinto	Douro.
51412	Arinto do Interior	Dão.
51413	Manteúdo	Alentejo.
51415	Uva Cão	Dão.
51417	Moscadet	Douro.
51513	Verdelho Roxo	Açores.
51514	Folha de Figueira	Beira Interior.
51516	Samarrinho	Douro.
51517	Cascal	Vinhos Verdes.
51602	Grangeal	Douro.
51604	Espadeiro Mole	Vinhos Verdes.
51606	Pilongo	Pinhel.
51608	Tinta Valdosa N	Douro.
51609	Dona Joaquina	Estremadura
51611	São Mamede	Vinhos Verdes.
51613	Rabigato Franco	Douro.
51617	Perrum	Algarve.
51701	Mourisco	Vinhos Verdes.
51708	Tinta do Rodo N	Douro.
51715	Praça	Douro.
51803	Preto Martinho	Douro.
51804	Monvedro	Dão.
51806	Verdelho Tinto	Vinhos Verdes.
51808	Beba	Algarve.
51816	Carrega Branco	Douro.
51901	Sousão	Vinhos Verdes.
51902	Vinhão	Vinhos Verdes.
51905	Tinta Caiada	Alentejo.
51910	Tamarez	Ribatejo.
51914	Síria	Beira Interior.
52002	Marufo	Beira Interior.
52003	Alfrocheiro	Dão.
52004	Cornifesto	Douro.
52005	Nevoeira	Douro.
52006	Patorra	Douro.
52007	Alvarinho	Vinhos Verdes.
52011	Rabo de Ovelha	Alentejo
52014	Rabigato	Douro.
52016	Bical	Estremadura
52017	Boal Espinho	Estremadura
52101	Tinta da Barca N	Douro.
52104	Arjunção	Algarve.
52105	Pedral	Vinhos Verdes.
52106	Rufete	Dão.
52111	Boal Vencedor B	Estremadura
52112	Gouveio	Douro.
52114	Alvadurão	Estremadura
52116	Boal Branco	Estremadura
52117	Dona Branca B	Dão.
52201	Tinta Carvalha	Douro.
52202	Negra Mole	Algarve.
52203	Ramisco	Estremadura
52205	Touriga Franca	Douro.
52206	Touriga Nacional	Dão.
52207	Encruzado	Dão.
52210	Terrantez	Dão.
52213	Loureiro	Vinhos Verdes.
52216	Trincadeira das Pratas	Ribatejo.
52301	Moreto	Alentejo.

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52304	Santareno	Douro.	52908	Amaral	Vinhos Verdes.
52306	Donzelinho Tinto	Douro.	52913	Galego Dourado	Estremadura
52307	Donzelinho Branco	Douro.	53006	Trincadeira	Douro.
52309	Boal Ratinho B	Estremadura	53013	Malvasia Rei	Douro.
52310	Avesso	Vinhos Verdes.	53015	Moscatel Nunes	Setúbal.
52311	Arinto	Bucelas.	53102	Primavera	E.A.N.
52313	Almafra	Estremadura	53103	Cabinda	E.A.N.
52314	Fonte Cal	Beira Interior.	53106	Castelão	Ribatejo.
52316	Antão Vaz	Alentejo.	53204	Amostrinha	Estremadura
52402	Camarate	Estremadura	53205	Malvasia Preta	Douro.
52407	Barcelo	Dão.	53206	Valbom	E.A.N.
52410	Cerceal Branco	Douro.	53207	Alvarelhão	Dão.
52412	Cercial	Bairrada.	53307	Tinto Cão	Douro.
52502	Tinta Francisca	Douro.	53308	Malvarisco	Setúbal.
52503	Jaen	Dão.	53312	Marquinhas	E.A.N.
52505	Benfica N	E.A.N.	53407	Mulata	E.A.N.
52506	Tinto Pegões	E.A.N.	53806	Roal	Setúbal.
52507	Batoca	Vinhos Verdes.	53807	Teinturier	Estremadura
52512	Malvasia Fina	Douro.	54006	Almenhaca	*
52513	Diagalves	Estremadura	54007	Alvar	*
52515	Jampal	Estremadura	54008	Alvar Roxo	*
52605	Carrasquenho	Estremadura	54009	Arinto Roxo	*
52606	Baga	Bairrada.	54010	Boal Barreiro	*
52612	Malvasia Fina Roxa	Dão.	54011	Branco João	*
52614	Vital	Estremadura	54012	Cainho	*
52615	Castelão Branco	Estremadura	54013	Calrão	*
52702	Parreira Matias	Estremadura	54015	Corval	*
52705	Preto Cardana	Ribatejo.	54016	Crato Espanhol	*
52706	Castelino	Estremadura	54017	Esgana Cão Tinto	*
52708	Folgasão Roxo	Beira Interior.	54018	Galego Rosado	*
52709	Folgasão	Douro.	54019	Leira	*
52710	Trajadura	Vinhos Verdes.	54020	Malvasia Romana	*
52714	Malvasia	Estremadura	54021	Malvia	*
52715	Viosinho	Douro.	54022	Perigó	*
52803	Bastardo	Douro.	54023	Pero Pinhão	*
52807	Borraçal	Vinhos Verdes.	54025	Pexem	*
52809	Azal	Vinhos Verdes.	54026	Rabo de Lobo	*
52810	Fernão Pires	Bairrada.	54027	Santoal	*
52815	Fernão Pires Rosado	Ribatejo.	54028	Zé do Telheiro	*
52902	Carrega Burros	Ribatejo.	54029	Tinta	*
52903	Rabo de Anho	Vinhos Verdes.	54030	Tinto Sem Nome	*
52904	Espadeiro	Vinhos Verdes.	54031	Valveirinho	*
52905	Tinta Barroca	Douro.	54032	Verdial Branco	*
52906	Tinta Grossa	Alentejo.	54033	Xara	*

* Recent Introduction in PRT051

Table 4. Autochthonous grapevine cultivars used in wine production in Portugal: Access number in the PRT051 collection, name of the grapevine cultivar, origin of grapevine accession.

Locus	N	Na	Ne	Ho	He	F
VVMD5	243	11	6.673	0.881	0.850	-0.036
VVMD7	243	12	3.965	0.765	0.748	-0.024
VVMD27	243	8	4.968	0.831	0.799	-0.041
VRZag 62	243	7	3.834	0.761	0.739	-0.030
VRZag79	243	12	4.051	0.765	0.753	-0.016
VVS2	243	15	5.822	0.881	0.828	-0.063

Table 5. Analyses of diversity in 243 Portuguese autochthonous cultivars: *locus*, accessions (*N*), number of alleles (*Na*), number of effective alleles (*Ne*), observed Heterozygosity (*Ho*), expected Heterozygosity (*He*) and Fixation Index (*F*).

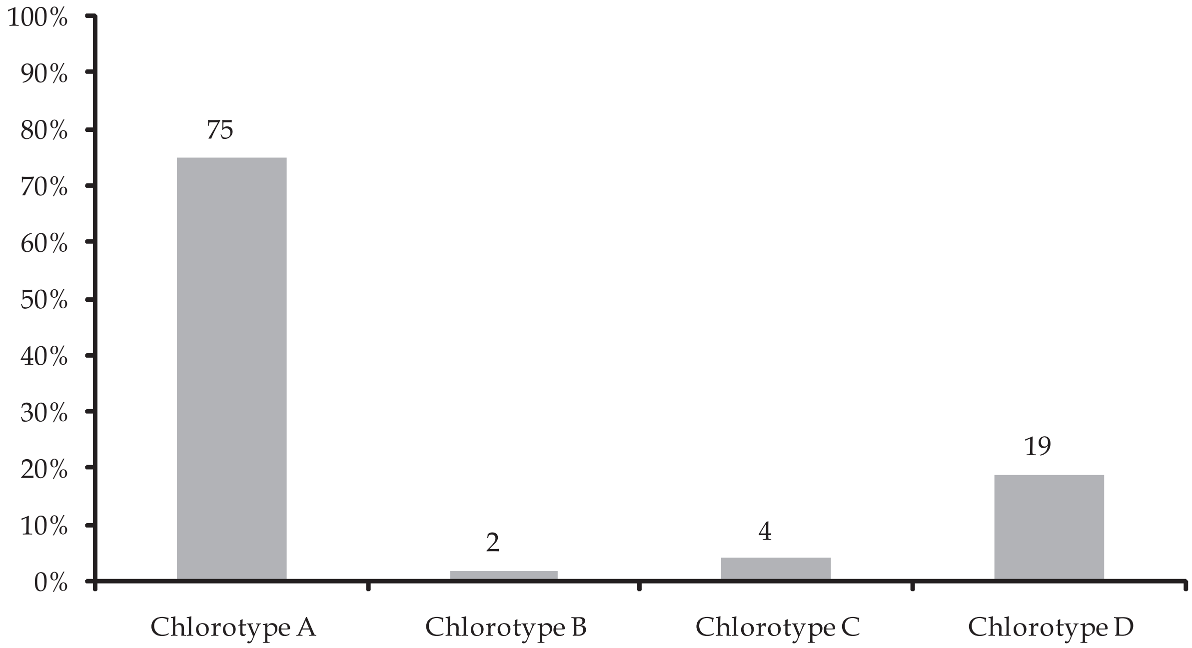

Figure 4. Chlorotypes of the Portuguese autochthonous grapevine cultivars. Chlorotype nomination according to [37].

The obtained results reinforce the suggestion that the Iberian Peninsula was a secondary center for grapevine domestication [37] despite the initial contribution of the Eastern gene pool some 3000 years ago and the more recent introgression from materials coming from central Europe.

Since 1978 a network of public and private associations lead by Antero Martins carried out an extensive work aiming at quantifying the intravarietal genetic variability within each of 45 Portuguese grapevine cultivars [54]. The static methods used were recently reviewed in [55]. These studies lead to the selection of a number of clones from Portuguese cultivars. In parallel and using the Geisenheim method of grapevine selection, a private nursery leaded by Jorge Böhm also selected a number of clones. Both groups registered a total of 122 clones from 27 different cultivars in the national grapevine catalogue (Table 6).

Variety	Obtainers		
	Plansel	UTL	INIAV
	clones JBP	clones ISA	clones EAN
Alfrocheiro T	41		
Alvarinho B	42; 43	44; 45; 46; 47	
Antão Vaz B	50		
Aragonez T	106; 110; 111; 114; 117		54; 55; 56; 57; 58; 59; 60
Arinto B	34; 35; 107		36; 37; 38; 39; 40
Bastardo T	48		
Bical B	119		
Castelão T	5; 25; 26		29; 30; 31; 32; 33
Cerceal Branco B	120		
Fernão Pires B	1		68; 69; 70; 71; 72; 73; 74
Gouveio B	121; 122; 123		
Jaen T		91; 92; 93; 94; 95; 96; 97	
Loureiro B		81; 82; 83; 84; 85	
Malvasia Fina B	127	98; 99; 100; 101; 102; 103; 104	
Moreto T	51		
Perrum B	128		
Sercial B	49; 105		
Síria B			75; 76; 77; 78; 79; 80
Tinta Barroca T	9; 129		
Tinta Caiada T	115; 116; 118		
Touriga Franca T	24		
Touriga Nacional T	16; 108; 112	17; 18; 19; 20; 21; 22; 23	
Trajadura B		86; 87; 88; 89; 90	
Trincadeira das Pratas B	124; 125; 126		
Trincadeira T	6; 7; 8; 109		10; 11; 12; 13; 14; 15
Vinhão T		61; 62; 63; 64; 65; 66; 67	
Viosinho B	53		
PE 1103 P	4		
PE 110 R	2		
PE 140 Ru	113		
PE 99 R 3	3		

Plansel/ JBP - Plansel (Wine and Nursery Company) / Jorge Böhm Plansel
UTL/ISA – Universidade Técnica de Lisboa / Instituto Superior de Agronomia
INIAV/ EAN – Instituto Nacional de Investigação Agrária e Veterinária/ Estação Agronómica Nacional

Table 6. List of the certified Portuguese clones of grapevine cultivars.

2.3. Overall diversity of the Portuguese grapevine germplasm

Portuguese wild vine populations are in an apparent geographic fringe of the species distribution but the country richness in cultivar diversity [8, 9] and the importance in allele contribution to the overall diversity of grapevine [56] tell another story. Figure 5 represents a Principal Coordinate Analysis of the diversity computed with the six nuclear SSRs used to genotype the 243 autochthonous cultivars and 53 wild vines, calculated with the program GenAlex6 [57] The two first coordinates represent 44.12% (1st coordinate - 24.08% and 2nd coordinate - 20.04%) of the total variance. Both subspecies are spread between the four quadrants although most wild vines are in the right quadrants. Even the plausible occurrence of feral forms cannot explain the overall dotting of the four quadrants since the alleles found in the wild vines population include private and particular alleles (data from [32]). When a Multiple Discriminant Analysis was used to assign the accessions to the different wild vine populations or to the cultivated group, most plants were correctly assigned and only three wild vines were assigned to the *vinifera* subspecies. On the other hand eight cultivars were assigned to the *sylvestris* subspecies [58]. This seems to corroborate the assumption that the part of the Portuguese germplasm was locally domesticated and contributes to the hypothesis that the Iberian Peninsula has been a secondary center for grapevine domestication [37].

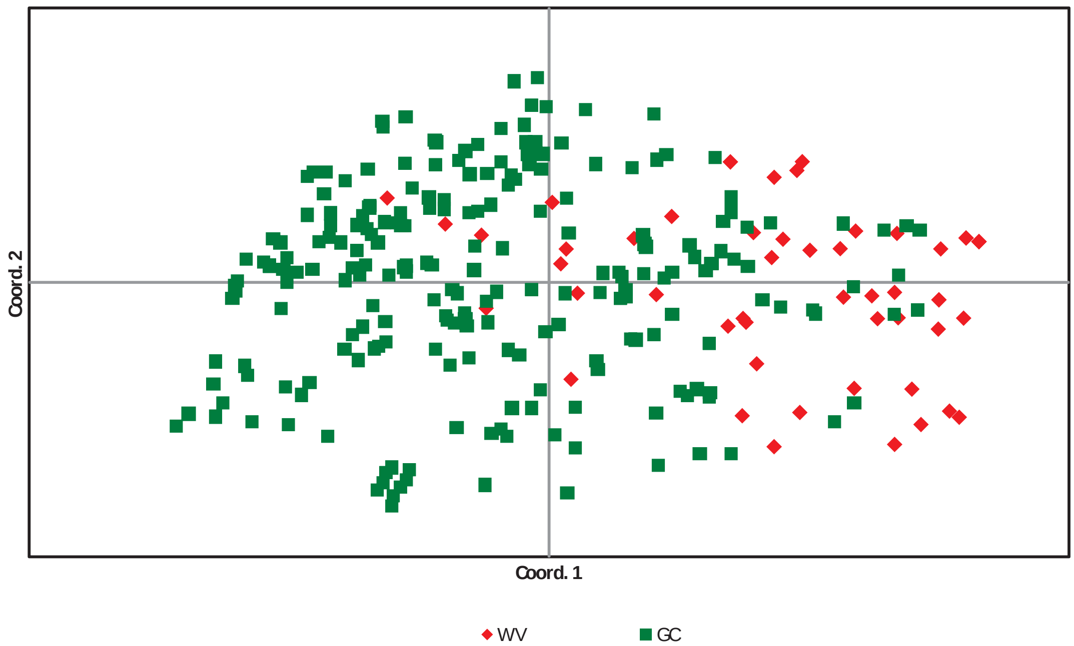

Figure 5. Scatter plot of a Principal Coordinate Analysis of six microsatellite loci from 243 Portuguese grapevine cultivars (GC, in green) and 53 wild vines (WV, in red) from four Portuguese populations.

3. The present situation of germplasm conservation in Portugal

Different strategies are needed to preserve the germplasm of the two grapevine subspecies. One obvious strategy is to maintain the natural habitats where the wild vines are present and keep them subjected to the selection pressures of the natural environment. For the cultivated subspecies the ideal situations should be maintaining the agro-systems where its diversity was buildup. However these *in situ* dynamic strategies must be accompanied by more static *ex situ* strategies, since natural habitats undergo a number of hazards and even the risk of disappearance, and today's commercial agro-systems tend to rely in a very small number of genotypes. Knowledge of the available diversity by multiple tools as reported above is the first step to decide on the strategies of conservation.

In situ conservation of wild vines populations is the leading choice to be considerate. There are a number of different problems that arise from this option: the land ownership where the plants subsist; the legal protection status of the subspecies; natural hazards, like fire; hazards caused by humans, like brutal cleaning of river banks; etc. Most of the populations are located in private owned land even when situated in areas where there is some kind of legal environment protection (populations 02 and 12). The first approach is to contact the land owner and explain the importance of wild vine populations and of the riparian habitats. In Portugal all contacted owners were willing to cooperate in the process of preserving the populations and some were even enthusiastic. Any major occurrence is usually reported like river bank cleaning or fire. Another important action is to contact the municipal authorities responsible for stream cleaning in order to adjust their actions to protect the riparian habitat. A good outcome of this policy was the case when the area where the population 04 inhabits was clean under the supervision of trained staff. Despite the positive results of these approaches some situations prove to be out of hand like the building of a dam, floods and fire. Population 03 was destroyed due to the construction of the Alqueva dam and part of population 12 was uprooted due to severe flooding. Populations 02 suffered a major fire in its habitat although with little loss in the total number of plants that recovered subsequently. To prevent the loss of the existing diversity an *ex situ* collection was started in 2005 at the CAN location (PRT051) with thirty wild vine accessions from three populations. Plants from other populations have been added to this collection.

Even though some European countries like France and Germany have a legal protection status for the subspecies *sylvestris*, in Portugal no such protection exists. An formal requirement was sent to the Portuguese agency for wildlife protection to establish a similar protected status for the Portuguese populations of *Vitis vinifera* subspecies *sylvestris* based on the information described in the previous sections.

Until the middle of the 20th century, most Portuguese farmers used to grow a mixture of vine cultivars as a way to overcome the effects of biotic and abiotic stresses but this situation was became increasingly rare and the vineyards are now mostly monovarietal. Nevertheless a recent report on *in farm* conservation, still found a considerable diversity in cultivated vineyards [59]. This is particularly observed when there is a weak relationship between the owner and the wine market, and a farm agro-ecological heterogeneity [59]. Today worldwide viticulture relies in a very restricted number of cultivars an even in a country like Portugal that has not abandoned its autochthonous cultivars, only 25 cultivars are planted in 80% of the new vineyards. The majority of the ancient cultivars is thus neglected and needs to be preserved *ex situ*.

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Ex situ collection of grapevine cultivars were settled initially in the 19th century after the arrival in Europe of *Dactylosphaera vitifoli*ae in order to be post philoxera repositories of local cultivars. Today two types of collections exist in Portugal: typical ampelographic collections (Table 7) and collections with a large number of different accessions of the same cultivar. These later were established as a result of a grapevine selection group network leaded by Antero Martins and today managed by PORVID - a public/private consortium. The methodology used to establish these collections was recently reviewed in [55].

Management	Owner	Coordinates Lat/Long	Number of accessions	Observations	International Code
INIAV	Public	39º 04′ N 9º 18′ W	754	in renovation	PRT 051
INIAV	Public	38º 41′ N 9º 19′ W	180	duplicate in PRT051	PRT 010
DRAPAlg Tavira	Public	37º 07′ N 7º 39′ W	129 wine 76 table	in renovation	PRT 068
DRAPN Santa Bárbara	Public	41º 10′ N 7º 33′ W	170		PRT 078
DRAPC Nelas	Public	40º 31′ N 7º 51′ W	65		PRT 079
DRAPC Lamaçais	Public	40º18' N 7º23' W	local cultivars		-
DRAPC Anadia	Public	46º26' N 8º26' W	local cultivars		-
DRAPN Sergude	Public	41º22'N 8º10'W	local cultivars		-
JMF, Wine Company	Private	38º 32′ N 8º 58′ W	439		-
ESPORÃO, Wine Company	Private	38º 23′ N 7º 33′ W	180	being installed	-
PORVID	Consortium	38º 38′ N 8º 38′ W	12	each variety with 300 clones	-
UTAD	Public	41º 17′ N 7º 44′ W	local cultivars		-
CVRVV	Public	41º48' N 8º24' W	local cultivars		-

Table 7. National and regional public and private ampelographic collections existing today.

The existing collections continue to perform several functions. These functions were initially related to the characterization and identification of cultivars using classic ampelography including: i) standardization of the morphological descriptors of *Vitis*; ii) morphological description of the cultivars iii) production of illustrate catalogues of cultivars iv) and sorting out synonyms and homonyms. These roles have evolved with the availability of new tools particularly the use of molecular markers that allowed the confirmation of suspected pedigrees and finding unsuspected ones. It also allowed tracing the remote history of grapevine domestication including the existence of several secondary domestication centers. The availability in one location of large number of genotypes of a highly heterozygous species also allow the development of genetic association studies like the one developed by Cardoso [60] that establish a candidate gene association with berry colour and anthocyanin content in 149 red and rose grapevine cultivars. Field performance of large numbers of cultivars in one spot as is the case of Esporão collection (Table 7) will help in the decision of what cultivar to plant and how to develop new wine types on the climate change scenario. Finnaly, morphological, molecular and field performance data will be useful in establishing core collections aiming a better management of the germplasm available.

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Author details

Jorge Cunha[1,2], Margarida Teixeira-Santos[3], João Brazão[1], Pedro Fevereiro[2,4] and José Eduardo Eiras-Dias[1]

1 INIAV, Quinta d'Almoinha, Dois Portos, Portugal

2 Universidade Nova de Lisboa, ITQB, Oeiras, Portugal

3 INIAV, Quinta do Marquês, Oeiras, Portugal

4 Universidade de Lisboa, Faculdade de Ciências, Lisboa, Portugal

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