

## Grapevine cultivar 'Alfrocheiro' or 'Bruñal' plays a primary role in the relationship among Iberian grapevines

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### Summary

The grapevine cultivar known in Portugal as 'Alfrocheiro', and in Spain as 'Bastardo Negro', 'Bruñal' or 'Baboso Negro', plays a central role in the genetic network of the Iberian Peninsula grapevine cultivars. Three sets of different molecular markers, SNPs, nSSRs and cpSSRs, revealed more than twenty parent-offspring links with this cultivar. 'Alfrocheiro' chlorotype is definitely Western European but their parents are still unknown. The distribution of the cultivar, their offspring as well as the two main co-parents direct to a geographic origin around the Portuguese and Spanish border. This cultivar and their progenies represent about 15 % of the total grapevine acreage in Portugal, stressing the importance of 'Alfrocheiro' in the Portuguese wine character.

Key words: 'Alfrocheiro'; pedigree; SNPs; nSSRs; cpSSRs.

### Introduction

'Alfrocheiro' is the prime name of the cultivar also known in Portugal as 'Tinta Francisca de Viseu', 'Tinta Bastardeira', 'Tinta Bastardinha do Douro' or, less frequently, 'Alfurcheiro'. This cultivar is also known in Spain as 'Albarin Negro', 'Albarin Tinto', 'Baboso Negro', 'Bruñal', 'Caiño Gordo' or 'Bastardo Negro' (<http://www.vivc.de/>). These synonymies have been confirmed by molecular markers (IBÁÑEZ *et al.* 2003, MARTÍN *et al.* 2003, 2006, ALMADANIM *et al.* 2007, GONZALES-ANDRÉS *et al.* 2007, VELOSO *et al.* 2010).

In Portugal the first written mentioning of 'Alfrocheiro' dates back to 1790 as a blue black cultivar grown in the vicinity of the city of Lamego, in the Douro wine area (LOBO 1790). In addition of its presence in the Douro region, FONSECA (1791) mentions 'Alfrocheiro' also in Bairrada and Estremadura in Portugal (cited in MENEZES 1896). In 1866 VILLA MAIOR states that it is also grown in the Trás-os-Montes (Portugal) wine area. In 1876 this cultivar was referenced in the Ampelografic Collection of the Botanical Garden of Coimbra University (VILLA MAIOR 1876, cited in MENEZES 1896).

A meticulous survey and the morphological characterization of the Portuguese cultivars done in the eighties of the 20<sup>th</sup> century show that this cultivar, in addition to its presence in the Douro wine area, was also cultivated in the Alentejo, Bairrada, Dão, Península de Setúbal, and Ribatejo-Oeste wine areas (IGEF 1984).

In Spain, the first mention to this cultivar is under the name of 'Albarín negro', by Suárez Cantón in 1879 (cited in CABELLO *et al.* 2011), in a publication about a wine from Asturias region, in Northern Spain. Later, in 1914, GARCÍA DE LOS SALMONES mentioned 'Albarin' cultivated in Galicia (Northwestern Spain) and 'Alvarin' in Asturias, and pointed out this cultivar as planted there before the phylloxera (*Dactylospira vitifoliae* Fitch) invasion.

'Alfrocheiro' is a recommended cultivar for many quality wine producing areas in Portugal: Alentejo, Bairrada, and Dão. It is also used for the production of regional wines in Alentejano, Beiras, Lisboa, Minho, Tejo and Terras do Sado (IVV 2013). In Spain, it is authorized in Protected Origin Denominations as 'Albarín Negro' or 'Bruñal' in the Northwestern regions (Arribes, Cangas, Castilla y León), and in the Canary Islands as 'Bastardo Tinto' or 'Bastardo Negro' (Abona, El Hierro, Gran Canaria, Islas Canarias, La Gomera, La Palma, Lanzarote, Tacoronte-Acentejo, Valle de Güimar, Valle de la Orotava, and Ycoden-Daute-Isora).

As an alternative to the well-established microsatellite markers (SSRs), Single Nucleotide Polymorphisms (SNPs) can also be used to study *Vitis* germplasm. They are abundant in the majority of genomes (BRUMFIELD *et al.* 2003), are less expensive and allele binning is simpler, making easier the interchange of genotypic information among different genotyping platforms and/or laboratories. SNP markers have been extensively used to identify cultivars, to saturate genetic maps and to study paternity relationships (LJAVETZKY *et al.* 2006; CABEZAS *et al.* 2011) to enlighten the genetic structure and the history of domestication of grapevine (MYLES *et al.* 2011).

'Alfrocheiro' was already identified as a progenitor of several cultivars either by SSRs (LACOMBE *et al.* 2013) or by SNPs (ZINELABIDINE *et al.* 2012) but until now their progenitors are unknown. The present study aims to clarify the relationships of 'Alfrocheiro' as a progenitor of more than 20 offspring's in the Iberian Peninsula, especially in Portugal.

### Material and Methods

**Plant material:** Young leaves of grapevine cultivars for DNA extraction were collected at the Portuguese National Ampelographic Collection (CAN), international code reference PRT051 (39°02'34.50"N and 9°10'56.94"W). All the studied cultivars involved in trios (parents and offspring) and duos (parent-offspring) relationships with 'Alfrocheiro' are listed in Tab. 1. Each cultivar studied is identified with the number and prime name reference used in the *Vitis* International Variety Catalogue (VIVC) (<http://www.vivc.de/>).

**DNA analyses:** DNA was isolated using the methodology described in LODHI *et al.* (1994). The 332 SNPs used were previously developed by LIJAVEZTKY *et al.* (2007) as described in CABEZAS *et al.* (2011) and genotyped at the Centro Nacional de Genotipado (CEGEN, [www.cegen.es](http://www.cegen.es)) according to ZINELABIDINE *et al.* (2012). Chloroplast SSRs were used to clarify which of the possible

parents was the maternal one, according to ARROYO-GARCIA *et al.* (2006). Twenty one nuclear SSRs were also used to confirm those new trios of parents and offspring with "Logarithm of odds" (LOD) scores below 60, following ALIFRAGKIS *et al.* (submitted). The SSRs and the cpSSRs were run in the automatic sequencer CEQ 8000 Genetic Analysis System (Beckman Coulter).

**Pedigree analyses:** Pedigree analysis was done using 1,117 profiles from the SNP database of the Instituto de Ciencias de la Vid y del Vino (ICVV), including 200 profiles from Portuguese cultivars collected at CAN (PRT051), to find the potential candidate parents. The SNP database of the ICVV includes genotypes from several different sources, mainly the ICVV grapevine collection (ESP217) and the Vitis Germplasm bank (VGB) from the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), in El Encín, (ESP080).

The software CERVUS 3.0 (KALINOWSKI *et al.* 2007) was used to obtain compatible trios and duos, and to calcu-

Table 1

List of cultivars studied, with name and accession codes, color of berry, number and prime name of the cultivar according to VIVC

Cultivar name	Accession nº	Colour of berry	VIVC number	VIVC prime name
Airén	ESP217-5000	B	157	Airen
Alfrocheiro Branco	PRT051-51610	B	8864	Douradinha
Alfrocheiro Preto	PRT051-52003	N	277	Alfrocheiro
Allarén	ESP080-0934	B		
Amaral	PRT051-52908	N	818	Amaral
Camarate	PRT051-52402	N	2018	Camarate Tinto
Casculho	PRT051-50901	N	14149	
Castelã	PRT051-51002	N	15672	
Castelão	PRT051-53106	N	2324	Castelao
Castelao Branco	PRT051-52615	B	2321	
Casteloa	PRT051-41303	N	23126	
Concieira	PRT051-50902	N	14144	
Cornifesto	PRT051-52004	N	2846	Cornifesto
Dona Branca	PRT051-52117	B	17676	Branda
Jaen	PRT051-52503	N	7623	Mencia
Jampal	PRT051-52515	B	5662	
Malvarisco	PRT051-53308	N	17249	
Malvasia Fina	PRT051-52512	B	715	Malvasia Fina
Malvasia Preta	PRT051-53205	N	15647	Malvasia Preta
Monvedro	PRT051-51804	N	17355	Monvedro
Monvedro de Sines	PRT051-41601	N	40729	
Moreto	PRT051-52301	N	7992	Moreto
Mourisco Branco	PRT051-50916	B	5335	Heben
Parreira Matias	PRT051-52702	N	15683	
Patorra	PRT051-52006	N	8977	
Ramisco	PRT051-52203	N	9899	Ramisco
Sarigo	PRT051-51316	B	5648	Cayetana Blanca
Tinta Gorda	PRT051-50607	N	8082	Mouraton
Tinta Grossa	PRT051-52906	N	40711	
Tinta Pomar	PRT051-50807	N	12493	
Tinto Cão	PRT051-53307	N	12500	Tinto Cao
Trincadeira das Pratas	PRT051-52216	B	15688	Trincadeira das Pratas
Gewürztraminer	ESP217-5083	B	12609	Gewurztraminer

B - white berry; N - black berry

late the corresponding LOD scores. Fourteen known pedigrees were used to empirically estimate the LOD threshold, which was set to 59 (Supplemental Tab. 1 in ZINELABIDINE *et al.* 2012).

### Results and Discussion

A subset of 48 SNPs developed by CABEZAS *et al.* (2011) allowed the identification of 191 distinct genotypes from the 200 Portuguese accessions. Together with those of the ICVV database, they account for 1,117 distinct genotypes (results not shown).

From the 332 SNPs scored, only 252 SNPs were informative to be used to establish the pedigrees (data not shown). The group of cultivars used for parentage analysis included trios previously detected by SSRs (BOWERS *et al.* 1999, CABEZAS *et al.* 2003, IBÁÑEZ *et al.* 2009, VARGAS *et al.* 2009, LACOMBE *et al.* 2013) and by SNPs (IBÁÑEZ *et al.* 2012, ZINELABIDINE *et al.* 2012). All genotypes involved in possible trios or duos (sharing at least one allele per *locus*) with 'Alfrocheiro' were selected (Tabs 1 and 2 and Figure). A total of twenty full possible trios and five duos were found (Figure).

From cpSSRs analyses no conclusion about the maternal line was possible since all the genotypes in the trios share the chlorotype A, the most common in the Western European gene pool (ARROYO-GARCIA *et al.* 2006).

Offspring of 'Alfrocheiro' and 'Cayetana Blanca': Four new trios were identified and six trios were confirmed (Tab. 2, Figure), where the parents

are 'Alfrocheiro' and 'Cayetana Blanca'. The genetic origin of 'Casculho', 'Castelã', 'Casteloa', and 'Jampal' is described for the first time, while the pedigrees of 'Camarate Tinto', 'Castelão', 'Cornifesto', 'Malvasia Preta', 'Moreto' and 'Mouraton' had been previously identified (ZINELABIDINE *et al.* 2012, LACOMBE *et al.* 2013). The known progeny of 'Alfrocheiro' and 'Cayetana Blanca' is thus increased to ten cultivars. The LOD score for these trios ranged from 69.0 to 88.2, higher enough to consider them reliable (Tab. 2).

'Cayetana Blanca' ('Sarigo') is a cultivar from the border between Extremadura (Spain) and Alentejo (Portugal). Among the ten offspring identified, only 'Mouraton' ('Tinta Gorda'), is planted in both Portugal and Spain. 'Mouraton' is an important cultivar for the Spanish wine production, but in Portugal it only exists in old vineyards in the Douro valley. 'Camarate', 'Castelão', 'Jampal', 'Malvasia Preta' and 'Moreto' are only planted in Portugal, with a considerable acreage especially in the south, being 'Castelão' the third most planted blue black cultivar of the country (IVV 2013). 'Casculho', 'Casteloa', and 'Cornifesto' cultivars are originally from the Douro valley in Portugal (MENEZES 1896) with around 500, 20 and 163 ha of planted area, respectively (BÖHM *et al.* 2007). No information exists about the origin of the cultivar 'Castelã', which is only found in collections.

Offspring of 'Alfrocheiro' and 'Hebén': 'Allarén', 'Castelão Branco', 'Trincadeira das Pratas', 'Malvasia Fina' and 'Tinta Grossa' are full siblings, descendants of 'Alfrocheiro' and the female variety 'Hebén'. The pedigree of the first three cultivars is described for the first time in this work. The pedigrees of 'Malvasia Fina' and

Table 2

Scheme of relationships involving the cultivar 'Alfrocheiro'

Offspring	Parent 1	Parent 2	Trio SNP compared	Trio LOD score	Comments
Parreira Matias	Alfrocheiro	Airén	211	75.7	
Douradinha	Alfrocheiro	Amaral	214	51.4	b)
Mouraton	Alfrocheiro	Cayetana Blanca	247	78.7	a)
Cornifesto	Alfrocheiro	Cayetana Blanca	240	70.3	a), b)
Camarate Tinto	Alfrocheiro	Cayetana Blanca	239	79.0	a), b)
Castelão	Alfrocheiro	Cayetana Blanca	250	86.4	a), b)
Malvasia Preta	Alfrocheiro	Cayetana Blanca	248	88.2	a), b)
Casteloa	Alfrocheiro	Cayetana Blanca	211	71.0	
Casculho	Alfrocheiro	Cayetana Blanca	212	69.0	
Castelã	Alfrocheiro	Cayetana Blanca	209	76.7	
Jampal	Alfrocheiro	Cayetana Blanca	218	76.4	
Moreto	Alfrocheiro	Cayetana Blanca	247	71.3	b)
Allarén	Alfrocheiro	Hebén	241	78.6	
Castelão Branco	Alfrocheiro	Hebén	220	71.5	
Malvasia Fina	Alfrocheiro	Hebén	252	70.9	b)
Tinta Grossa	Alfrocheiro	Hebén	222	74.7	b)
Trincadeira das Pratas	Alfrocheiro	Hebén	245	79.9	
Mencía	Alfrocheiro	Patorra	249	64.7	
Conceira	Alfrocheiro	Ramisco	214	54.9	
Malvarisco	Alfrocheiro	Tinto Cão	202	57.7	

a) Confirmation of prior publication: ZINELABIDINE *et al.* (2012), using SNPs.

b) Confirmation of prior publication: LACOMBE *et al.* (2013), using SSRs.

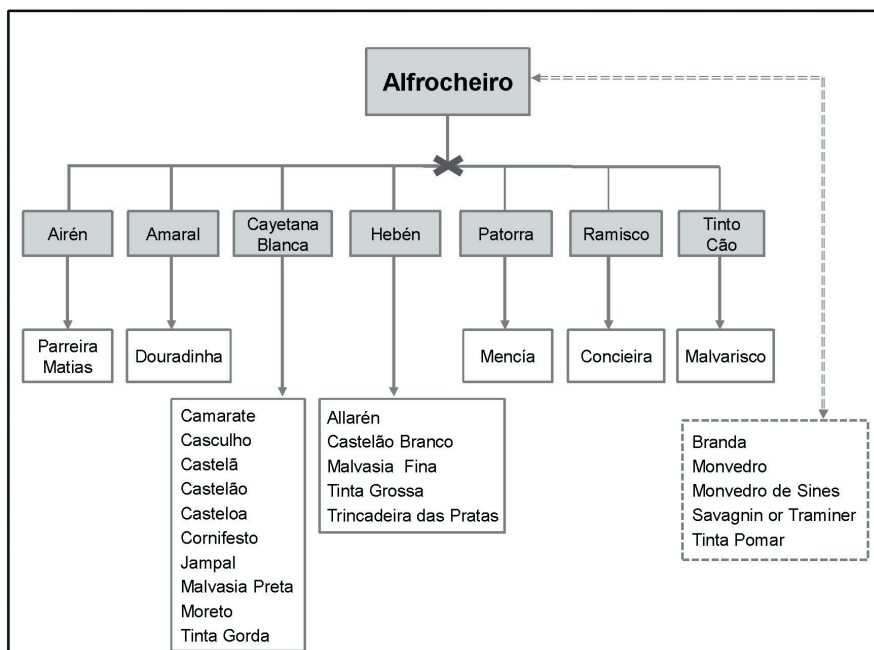


Figure: Genetic relationship of cultivar 'Alfrocheiro'. Grey background and solid lines indicate full pedigree (trio, parents and offsprings), while broken lines indicate cultivars sharing at least one allele per locus for 252 SNPs analyzed (possible parent-offspring relationship).

'Tinta Grossa' had previously been identified by LACOMBE *et al.* (2013), using SSRs. The LOD score for these trios was also high, and ranged between 70.9 and 79.9 (Tab. 2).

'Hebén' is an important Spanish variety already mentioned in the classical work of HERRERA (1513), and it has a very large progeny (ZINELABIDINE *et al.* this issue). It is cited by ALARTE in 1711 with its Portuguese name 'Mourisco Branco'. The name 'Mourisco' (literally Moorish) is often used in the Portuguese rural vocabulary to refer to an ancient variety.

'Malvasia Fina' (with more than 20 synonyms, the most frequent being 'Boal', 'Boal Cachudo' and 'Boal da Madeira' in Portugal, and 'Torrantes' and 'Gual' in Spain) is an important white cultivar for many wine regions in Portugal, covering 2,121 ha (IVV 2013) especially at the Douro and Porto Protected Denomination of Origin (PDO) and in the Madeira Island, where it is known as 'Boal'. In Spain it covers 141 ha (2009), mostly in Galicia, (CABELLO *et al.* 2011), although it is also authorized in several Canarias PDO. This cultivar was also identified as the progenitor of Ratinho (IVC\_9927) (LOPES *et al.* 1999).

'Castelão Branco' and 'Tinta Grossa' cultivars are at present only planted in Portugal in 30 and 100 ha, respectively, although written records could be found prior to 1896 (BÖHM *et al.* 2007). 'Trincadeira das Pratas' is planted also in Portugal on 270 ha in Alentejo and Tejo PDOs (BÖHM *et al.* 2007), but references to this cultivar are just found in the 20<sup>th</sup> century. 'Allarén' is a minor cultivar from Spain (CABELLO *et al.* 2011), almost extinct, and found in Leon region. It is not known in Portugal. According to the IVC, 'Allarén' and 'Trincadeira das Pratas' are synonyms, but SNP data show they are definitely different, even if no more than one sample of 'Allarén' was studied.

Other trios involving 'Alfrocheiro': Four new trios were identified from crosses between 'Al-

frocheiro' and four known cultivars: 'Airén', 'Patorra', 'Ramisco', and 'Tinto Cão' (Tab. 2, Figure). Another trio, previously identified by LACOMBE *et al.* (2013) with SSRs, was confirmed: 'Douradinha' is the progeny of 'Alfrocheiro' and 'Amaral'.

'Alfrocheiro' and 'Airén' are the progenitors of 'Parreira Matias'. 'Airén' is an old and widely planted cultivar, typical of Castilla La Mancha (Spain). Curiously no written mention for this cultivar exists in Portugal and it could not even be found in old vineyards (IGEF 1984). 'Parreira Matias' was traditionally planted in the Lisbon area and was important in the historical Colares wine (LAPA 1866), the only region in Portugal that survived the 19<sup>th</sup> century phylloxera devastation and where, until today, grapevines are planted on their own roots. Currently 'Parreira Matias' is almost extinct and it vanished from Colares PDO.

'Alfrocheiro' and 'Patorra' are the parents of 'Mencia'. 'Patorra' is mentioned in a survey done in 1865 and was planted in Alijó county in the Portuguese Douro valley (MENEZES 1900). Today it is a minor cultivar covering only 20 ha (BÖHM *et al.* 2007). 'Mencia' ('Jaen' in Portugal) is a recommended cultivar for all Northern Spanish wine areas, including all the PDO in Galicia and several in Castilla and Leon, being of special relevance in the PDO Bierzo. The first mention to this variety in Spain was found in 1914 (GARCIA DE LOS SALMONES 1914), and in 2009 there were more than 8,000 ha cultivated. In Portugal, at the Dão PDO and Beiras Protected Geographical Indication (PGI) 'Mencia' ('Jaen') is cultivated on 1,731 ha (IVV 2013). In the mentioned 1865 survey, it was already referred to be grown in the Viseu county (Dão PDO), where it is still very important.

'Alfrocheiro' and 'Ramisco' are the progenitors of 'Concieira' with a LOD score 54.9. Although this score is under 60, the genotyping with 21 SSRs (Tab. 3) supports

Table 3

Genetic profile with 21 SSRs used to confirm the full trios with LOD score under 60. Allele sizes are given in base pairs

Locus	Allele	Parent 1	Offspring	Parent 2	Parent 1	Offspring	Parent 2
		Alfrocheiro	<b>Concieira</b>	Ramisco	Alfrocheiro	<b>Malvarisco</b>	Tinto Cão
VVMD 27	1	177	<b>165</b>	179	177	183	179
	2	187	179	183	187	187	183
VVMD 25	1	249	239	239	249	241	241
	2	255	249	239	255	249	241
VVMD 28	1	237	229	229	237	249	255
	2	249	249	255	249	267	267
VVMD 32	1	251	<b>259</b>	251	251	249	249
	2	271	271	253	271	271	271
VVMD 62	1	186	186	186	186	192	184
	2	198	198	294	198	198	192
VVMD 5	1	223	235	223	223	223	229
	2	235	235	235	235	229	231
VVMD 7	1	251	237	237	251	251	237
	2	255	251	261	255	261	261
VVS 2	1	141	131	131	141	131	131
	2	149	149	155	149	149	131
VMC7h3	1	134	134	134	134	134	134
	2	138	138	134	138	134	138
VMC4f3	1	180	172	172	180	172	172
	2	206	206	178	206	178	206
VVMD 24	1	207	207	207	207	211	211
	2	211	211	211	211	211	211
VVIb01	1	290	290	292	290	290	290
	2	295	308	308	296	290	290
VVIIn73	1	262	264	258	262	262	262
	2	264	264	264	264	264	264
VVIh54	1	161	<b>157</b>	161	161	161	141
	2	165	165	165	165	165	165
VVIq52	1	86	86	86	86	82	82
	2	86	86	86	86	86	82
VVIp31	1	179	179	191	179	175	175
	2	189	191	191	189	189	189
VVIp60	1	305	317	305	305	313	313
	2	317	317	317	317	317	321
VVMD21	1	246	246	240	248	240	240
	2	254	254	246	254	254	254
VMC1b11	1	166	166	166	166	172	172
	2	172	172	172	172	174	174
VVIIn16	1	153	151	143	153	153	153
	2	159	153	151	159	157	157
VVIv67	1	370	362	358	370	356	356
	2	374	370	362	374	370	356

Note: The trio 'Alfrocheiro'-'Douradinha'-'Amaral' was not studied further because it had been previously published by LACOMBE *et al.* 2013. Numbers in bold and italic are incompatible alleles, probably due to mutation.

this trio. 'Ramisco' is the cultivar that gives the distinctive astringent character to Portuguese Colares wines allowing them to age for many years. It is mentioned since at least 1866 (LAPA 1866). Today there are around 45 ha planted in Portugal with 'Concieira' (BÖHM *et al.* 2007) and no written mentioning of this cultivar could be found.

'Alfrocheiro' and 'Tinto Cão' are the progenitors of 'Malvarisco' with a LOD score 57.7 (Tab. 2). Again, even though this score is under 60, the genotyping with 21 SSRs

(Tab. 3) supports this trio. Carvalho in 1771 refers 'Tinto Cão' as an important cultivar from the Portuguese Douro wine area (MENEZES 1896), a position still maintained today. 'Malvarisco' is a minor cultivar (1.7 ha) (BÖHM *et al.* 2007) and apart from the place where it was found, in the Setúbal area (IGEF 1984), there is no further information about the cultivar.

SNP analysis confirmed the results previously obtained by LACOMBE *et al.* (2013) for the cultivars 'Alfro-



cheiro' and 'Amaral' as parents of 'Douradinha'. The LOD score obtained with SNPs was below 60 (Tab. 2), but the previous analysis with 20 SSRs supports this trio (LACOMBE *et al.* 2013). 'Amaral' is mentioned in the classical work of Fernandes published in 1532 (MENEZES 1896) and today is mostly planted in the Northwest of the Iberian Peninsula either in the Vinhos Verdes (Portugal) or, known as 'Caiño Bravo', in Galicia (Spain). 'Douradinha' is mentioned as cultivar from the Douro (Portugal) in the work of Aguiar from 1866 (cited in MENEZES 1896). This cultivar is called 'Alfrocheiro Branco' in the Dão PDO, which hints to one of its parents.

'Alfrocheiro' possible first degree relationships: Five cultivars - 'Branda', 'Monvedro', 'Monvedro de Sines', 'Tinta Pomar' and 'Savagnin' (or 'Traminer' or 'Gewürztraminer') - share one SNP allele per locus with 'Alfrocheiro', and thus have a possible parent-offspring relationship (Figure).

The adoption of the name 'Branda' in 2012 (PORTARIA n° 380/2012) was triggered by the widespread use of 'Dona Branca' (literally white lady) to name several different white genotypes in a typical case of homonymy. There is even a different Spanish genotype homonym named 'Doña Blanca'. This genotype was collected in the Portuguese Dão PDO (192 ha, BÖHM *et al.* 2007) where is referenced since 1866 (AGUIAR 1866, cited in MENEZES 1896).

'Monvedro' (VIVC\_17355) was also collected in the Dão PDO and 'Monvedro de Sines' (PRT051\_41601, VIVC\_40729) was collected in Peninsula de Setúbal PDO. The official Portuguese name of 'Monvedro de Sines' is 'Bonvedro'. There are other two homonyms of Monvedro in Portugal. 'Monvedro' from Algarve, a synonym of 'Trincadeira' (VIVC\_15685), 'Monvedro' collected at Bucelas with the official Portuguese name 'Tinta Caiada', that is a synonym of the Spanish 'Parraleta' (VIVC\_8951). These four non redundant genotypes were profiled by SSRs in VELOSO *et al.* (2010). LACOMBE *et al.* 2013 also identified a first degree relationship between 'Alfrocheiro' and the Vassal accession 'Monvedro de Sines' (# 3371). MENEZES (1900) mentioned only one 'Monvedro' as a cultivar frequently planted in Aveiro, Lisboa and Faro (Algarve) regions. Today both two cultivars 'Monvedro' and 'Bonvedro' (= 'Monvedro de Sines') are two minor cultivars with 8 ha and 3 ha planted in Portugal, respectively (BÖHM *et al.* 2007).

'Tinta Pomar' was referred in the Ampelographic Collection of the Botanical Garden of Coimbra University in 1877 by VILLA MAIOR (1877) (cited in MENEZES 1896). Today it is a minor cultivar from the Portuguese Douro wine region where it occupies around 70 ha (BÖHM *et al.* 2007).

Finally there is a possible parent-offspring relationship between 'Alfrocheiro' and 'Savagnin'. 'Savagnin' is a very old cultivar which has also many progenies spread all over Europe, and especially in the Northwestern region of the Iberian Peninsula like 'Gouveio' or 'Verdejo' (LACOMBE *et al.* 2013). The absence of full trios in these five cases prevents the precise establishment of which cultivar is the parent and of which is the offspring, but the older historical references to 'Savagnin' point out that 'Alfrocheiro' could

be the offspring of 'Savagnin', and the parent of the other three cultivars. 'Savagnin', with chlorotype D (www.vivc.de) would be the male progenitor since 'Alfrocheiro' bears chlorotype A.

The distribution of 'Alfrocheiro' and its offspring, as well as of its two main co-parents ('Cayetana Blanca' and 'Hebén'), point to a geographic origin around the Portuguese and Spanish border. This cultivar and its progenies represent around 15 % of the Portuguese vineyards, stressing its importance in the Portuguese wine character. Despite being referred as having a low intra-varietal variability (GONÇALVES 1996), 'Alfrocheiro' is definitely a very old cultivar and most of its important offspring e.g. 'Castelão', 'Camarate', 'Jampal', 'Malvasia Fina', 'Mencía' and 'Moreto' are also referenced before or during the phylloxera pest outbreak of 1851.

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