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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 20th 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 (*Dactyloshpaera 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) 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.

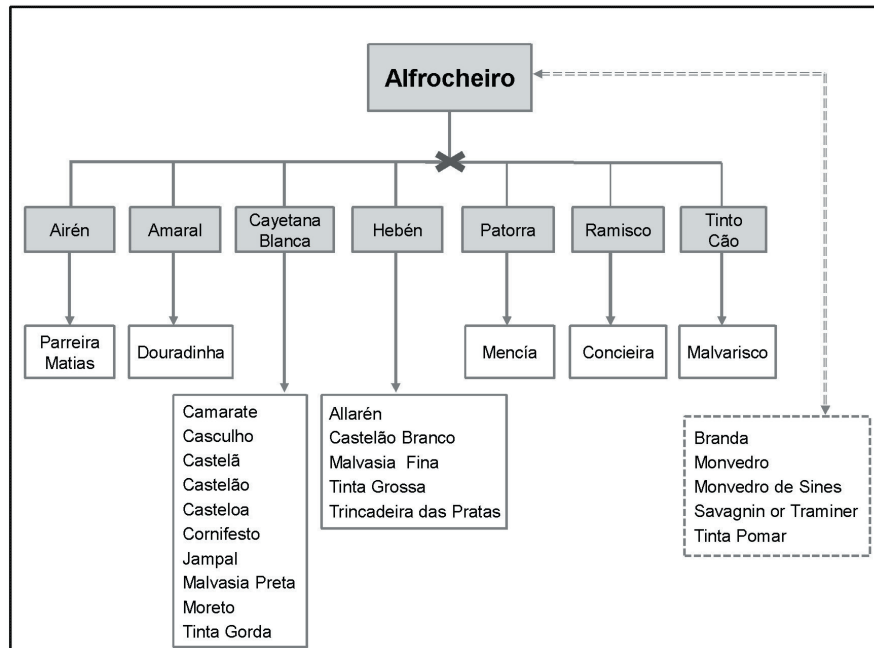


Figure: Genetic relationship of cultivar 'Alfrocheiro'. Grey background and solid lines indicate full pedigree (trio, parents and offsprings), while broken lines indicate cultivars sharing at least one allele per locus for 252 SNPs analyzed (possible parent-offspring relationship).

'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 20th 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 19th 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 | Concieira | Ramisco | Alfrocheiro | Malvarisco | Tinto Cão |
| VVMD 27 | 1 | 177 | 165 | 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 | 259 | 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 | 157 | 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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