

## Identification of minority grapevine cultivars from Vinhos Verdes Portuguese DOC Region

V. FERREIRA<sup>1)</sup>, O. PINTO-CARNIDE<sup>1)</sup>, T. MOTA<sup>2)</sup>, J. P. MARTÍN<sup>3)</sup>, J. M. ORTIZ<sup>3)</sup>, and I. CASTRO<sup>1)</sup>

<sup>1)</sup> Instituto de Biotecnologia e Bioengenharia, Centro de Genómica e Biotecnologia, Universidade de Trás-os-Montes e Alto Douro (IBB/CGB-UTAD), Vila Real, Portugal

<sup>2)</sup> Estação Vitivinícola Amândio Galhano/ Comissão de Viticultura da Região dos Vinhos Verdes (EVAG/CVRVV), Arcos de Valdevez, Portugal

<sup>3)</sup> Dpto. de Biotecnología - Biología Vegetal, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Madrid, Spain

### Summary

**The Portuguese Vinhos Verdes DOC Region retains a considerable biodiversity in grapevine varieties. Unfortunately, regional grapevine patrimony is in danger of disappearing. The present study was carried out in order to genotype, using 12 microsatellite loci, a group of 39 accessions of Vinhos Verdes DOC region, including underuse and minor varieties included in the wine list of varieties and also several unknown accessions with regional designations. The accessions analyzed were identified and grouped into 34 different genotypes, nine of them referred as new genotypes. Some new synonyms were detected, namely between Spanish and Portuguese cultivars. Misidentifications and wrong designations were also detected.**

**Key words:** Autochthonous cultivars; grapevine germplasm; cultivar identification; microsatellites; molecular markers.

### Introduction

Vinhos Verdes is an important wine-producing DOC Region, located in northwest of Portugal, which comprises 13 % of the Portuguese area under vineyard (IVV 2013).

During the process of the regional viticulture, only high quality varieties have been considered for wine production, leading to a gradual depreciation of varieties grown on a small scale, that were included in official lists as secondary varieties (MOTA *et al.* 2013). Therefore, the production of Vinhos Verdes DOC wine with sub-region designation is presently restricted to 15 cultivars (Ministerial Order 668/2010). Thirty other varieties (including 16 exclusive of Vinhos Verdes) can be used in the production of Vinhos Verdes DOC wine, but are excluded of the production of local and typical wines, embraced in the classification "sub-regional".

Nevertheless, in the beginning of the XX<sup>th</sup> century around one hundred different names of grapevine cultivars were referenced in the restricted area of some municipalities of Vinhos Verdes Region (BRAVO and OLIVEIRA 1916). After a rigorous prospection of autochthonous varieties in the 80s, at this moment the existence of some of them is

confined to collection fields. The great diversity and rich density of Vinhos Verdes Region in autochthonous varieties is in danger of disappearing and, consequently, dangerously shrinking grapevine genetic pool and increasing crop vulnerability to climate changes, new pests and diseases (ALMADANIM *et al.* 2007).

There are still diverse grapevine synonymies and homonymies to clarify that, together with the existence of unnamed accessions, are a source of misidentification and confusion in grapevine varieties designations (MARTÍN *et al.* 2011), constituting one of the most challenging and unfinished problems for viticulture worldwide and germplasm management (VELOSO *et al.* 2010).

The goals of this study were to undertake an identification of accessions that include a broad representation of autochthonous and minor grapevine cultivars from Vinhos Verdes DOC Region, using 12 microsatellite loci. Additionally, we tried to investigate the origin of this material through amplification of three cpSSR loci. In the scope of this study, the detection of possible synonymies, homonymies, and eventually new genotypes, will solve problems concerning the management of grapevine field collections, as well as contribute to an efficient preservation of old local germplasm that represents valuable genetic combinations for a new and renewed viticulture.

### Material and Methods

A total of 39 accessions were collected in an ampelographic collection of the viticulture center "Estação Vitivinícola Amândio Galhano" (EVAG) located in Arcos de Valdevez (41°48'N; 8°25'W), in the middle of *Vinhos Verdes* DOC Region.

For each accession, total DNA was extracted from wood material using the "DNeasy® Plant Mini Kit" (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Amplification and analysis of the 12 microsatellite loci VVMD5, VVMD7, VVMD27, ssrVrZAG62, ssrVrZAG79, VVMD28, VVMD32, VVS2 (BOWERS *et al.* 1999), VVIv37, VVIv67, VVIp31 and VMC4f3, was carried out as previously described (CASTRO *et al.* 2011). Genotyping results, obtained by the amplification of 12 SSR loci were compared with publicly available databases of

microsatellite profiles (<http://sivvem.monbyte.com/>; <http://www.eu-vitis.de/index.php>).

Three chloroplast SSR loci (ccmp3, ccmp5 and ccmp10), described as the most polymorphic in grapevine, were analyzed in all distinct genotypes using the consensus primer pairs designed by WEISING and GARDNER (1999) as described in CASTRO *et al.* (2013).

Ampelographic characterization was carried out in five plants of each accession with 12 OIV primary descriptors (001; 004; 016; 051; 067; 068; 070; 076; 079; 081-2; 084; 087) (OIV 2009), including both qualitative and quantitative characteristics, observed or measured in 10 shoots and 10 leaves.

## Results and Discussion

In this work, many of the sampled accessions of Vinhos Verdes DOC Region were collected either as unidentified or with local names. As a result of the accession characterization, the presence of synonymies or homonymies and some identification were achieved. Twenty seven out of the 39 accessions analyzed with the 12 SSR loci were coincident with what has been previously characterized by VELOSO *et al.* (2010) (Tab. 1). From these 27 accessions, 15 are in catalogue and 15 are officially considered as minority cultivars (Tab. 1).

Identification, synonymies and errors of denomination: Unidentified accessions such as 'Carvalho 1' and 'Carvalho 2' were identified as 'Trincadeira' (also known as 'Tinta Amarela' in Portugal) and as 'Malvasia Preta', respectively (VELOSO *et al.* 2010).

SSR loci amplification also allowed the identification of other accessions, namely, 'Touriga Tinto' accession that revealed to be the official cultivar 'Jaen' having 'Mencia' as synonym in Spain. Ampelographic analysis confirmed this result once all 12 OIV descriptors analyzed agree with am-

pelographic characterization of 'Jaen'. 'Cainho de Moreira' accession was identified as the official Portuguese cultivar 'Cainho', having 'Caiño Blanco' as synonymy in Spain.

Several new synonymies were also found: 'Verdeal' accession revealed to be 'Melhorio' cultivar, coinciding in both molecular and ampelographic characterization, being considered a new synonymy of the official cultivar 'Melhorio'.

'Verdelho Tinto' and 'Verdelho Feijão' accessions revealed the same SSR profile, which corresponds to the 'Verdelho Tinto' genotype described by VELOSO *et al.* (2010). On this way, 'Verdelho Feijão' represents a synonymy of 'Verdelho Tinto' cultivar in Vinhos Verdes DOC Region. Despite SSR profile of both accessions match in all 12 SSR loci, these two accessions differ in two mature leaf morphologic characters (Figure A). 'Espadeiro Geme' corresponds to the genotype of 'Espadeiro Mole', described by VELOSO *et al.* (2010), and to the Vinhos Verdes cultivar 'Espadeiro Mole' (CASTRO *et al.* 2011), also analyzed in this study. This profile also coincides with the one published by MARTIN *et al.* (2006), for the Galician 'Férron' cultivar, that is a synonymy of the cultivar 'Caiño do Freixo' in Spain (SANTIAGO *et al.* 2005).

'Cainho Branco' and 'Galeguinho' accessions revealed the same SSR profile, matching with the profile of 'Alvarinho' cultivar in VELOSO *et al.* (2010) and 'Alvarinho' of Vinhos Verdes DOC Region described by CASTRO *et al.* (2011).

Three synonymies between Portuguese and Spanish cultivars were also identified for the first time. 'Doçar' accession, that revealed to be the official cultivar 'Doçal' (VELOSO *et al.* 2010), has the same SSR profile of the Spanish 'Corbillón' cultivar (DÍAZ-LOSADA *et al.* 2011a). 'Formosa' and 'Mourisco' accessions also showed undescribed synonymies with the Spanish cultivars 'Mantúo' (personal information) and 'Tinta Castañal' (SANTIAGO *et al.* 2008), respectively. 'Tinta Castañal' typically grows in Galicia and

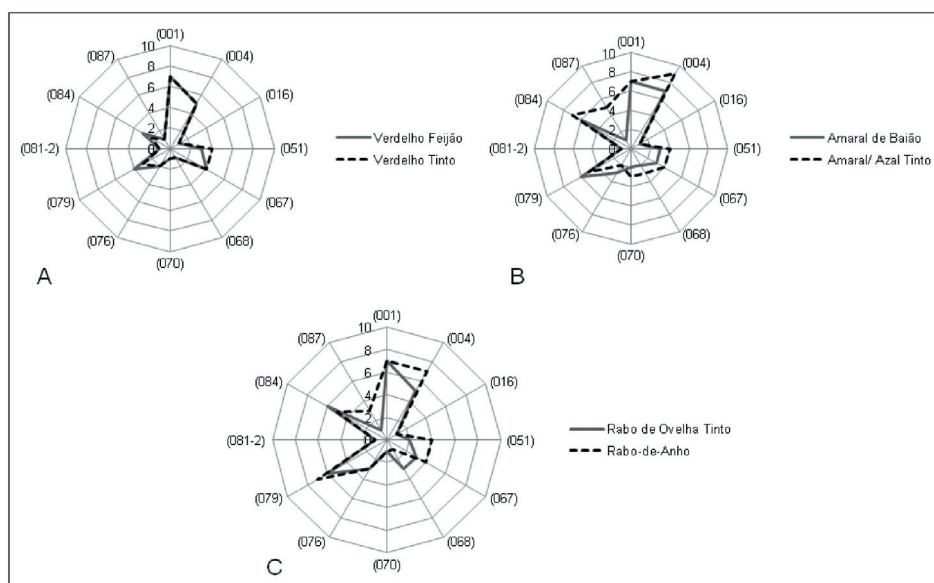


Figure: Graphic representation of the ampelographic characterization based on 12 descriptors of the "Primary descriptor priority list" suggested by the OIV. A: 'Verdelho Feijão' vs. 'Verdelho Tinto'; B: 'Amaral de Baião' vs. 'Azal Tinto'/'Amaral'; C: 'Rabo de Ovelha Tinto' vs. 'Rabo de Anho'.

Table 1

Grape material studied with respective origin, berry colour, SSR identification with recommended names and synonymies in Portugal and Spain

Accession local name	Origin (Parish/Municipality)	Berry colour	SSR identification	Reference
Amaral de Baião	Sta Marinha de Zêzere/Baião	B	New genotype	--
Amaral/ Azal Tinto	Oleiros/Ponte da Barca	B	Amaral (PT)◇; Caiño Bravo (SP)	a; e; f
Azal Sto. Tirso	Refojos/Sto. Tirso	W	New genotype	--
Bogalhal	Gagos de Cima/ Celorico de Basto	B	New genotype	--
Branjo	Gatão de Baixo/ Amarante	B	Branjo	d
Carvalho 1	Antede/ Baião	B	Trincadeira; Tinta amarela	d
Carvalho 2	Antede/ Baião	B	Malvasia Preta	d
Cainho da Gemieira	Gemieira/Ponte de Lima	W	Alvarinho (PT)◇; Albariño (SP)	d; e; f
Cainho de Moreira	Moreira/ Ponte de Lima	W	Cainho (PT)*◇; Caiño Blanco (SP)	b; e
Cascal	Friande/Felgueiras	W	Cascal*◇	d
Doçália	Marrancos/ Vila Verde	B	New genotype	--
Doçar	Moreira/ Ponte de Lima	B	Doçal (PT) *◇; Corbillón (SP)	d; g
Doce	Moreira/ Ponte de Lima	B	Doce*	d
Esgana Cão	Oleiros/ Ponte da Barca	W	New genotype	--
Esganinho	Moreira/ Ponte de Lima	W	Esganinho*◇	d
Esganoso	Moreira/ Ponte de Lima	W	Esganoso*	d
Formosa	Antede/ Baião	W	Mantúo (SP)	h
Espadeiro Mole	Pias/Monção	B	Espadeiro Mole (PT)*◇; Férron (SP)	b; d; e
Espadeiro Gême	Gême/Vila Verde	B	Espadeiro Mole (PT)*◇; Férron (SP)	b; d; e
Galego	Pico de Regalados/Vila Verde	B	Galego	d
Galeguinho	Moreira/ Ponte de Lima	W	Alvarinho (PT)◇; Albariño (SP)	d; e; f
Labrusco I	Santiago de Piães/Cinfães	B	Labrusco*◇	d
Labrusco II	Antede/ Baião	B	Labrusco*◇	d
Lameiro	Sampriz/Ponte da Barca	W	Lameiro*◇	d; e
Loureiro Bravo	Queijães/ Fafe	B	New genotype	--
Melhorio	Gagos de Cima/ Celorico	B	Melhorio*	d; e
Mourisco	Goães/ Vila Verde	B	Mourisco (PT)*◇; Tinta Castañal (SP)	c; d
São Mamede	Oleiros/ Ponte da Barca	W	São-Mamede*◇	d
Sezão	Faia/ Arco de Baulhe	B	Sezão◇	d
Sousão Galego	Gagos de Cima/ Celorico	B	New genotype	--
Pintosa/Branco Escola	Sto. Tirso	W	Pintosa*◇	d
Rabo de Ovelha Tinto	Antede/ Baião	B	New genotype	--
Tinta Varejoa	Coleção da Casa do Douro	B	Varejoa	d
"Touriga" Tinto	Antede/ Baião	B	Jaen (PT)◇; Mencia (SP)	d
Transâncora	Sta Maria de Zêzere/ Baião	B	Transâncora*	d
Verdelho Feijão	Facha/Ponte de Lima	B	Verdelho Tinto*◇	d
Verdelho Quelho	Bairros/Castelo de Paiva	B	New genotype	--
Verdelho Tinto	Sampriz/ Ponte da Barca	B	Verdelho Tinto*◇	d
Verdeal	Gagos de Cima/ Celorico de Basto	B	Melhorio*	d; e

\* Minority cultivar; ◇ In the list of suitable varieties for wine production; PT: Portugal; SP: Spain; a: PINTO-CARNIDE *et al.* (2003); b: MARTIN *et al.* (2006); c: SANTIAGO *et al.* (2008); d: VELOSO *et al.* (2010); e: CASTRO *et al.* (2011); f: DÍAZ-LOSADA *et al.* (2011b); g: DÍAZ-LOSADA *et al.* (2011a); h: personal information.

its synonym is 'Rabo de Cordeiro' (GARCÍA DE LOS SALMONES 1912).

Errors of denomination were also reported. 'Amaral de Baião' accession revealed a different SSR profile from the official cultivar 'Amaral' (PINTO-CARNIDE *et al.* 2003, CASTRO *et al.* 2011). On the other hand, we demonstrated that the accession 'Amaral/Azal Tinto' has the same profile of the official 'Amaral' cultivar. Considering the fact that both accessions have 'Amaral' in their designations, ampelographic characterization was also performed. Morphological results are in agreement with molecular data, once both accessions differ in nine of the 12 OIV descriptors

analyzed, being in fact two different accessions (Figure B). Possible crosses and descendents involving 'Amaral' cultivar were also identified, proving its previously described ancestry in the Northwest of the Iberian Peninsula (CASTRO *et al.* 2012).

Another wrong denomination is the case of 'Rabo de Ovelha Tinto' accession. Officially, 'Rabo de Ovelha Tinto' is the 'Rabo de Anho' cultivar. On this way, one would expect that the SSR profiles of 'Rabo de Ovelha Tinto' accession would correspond to 'Rabo de Anho' cultivar analyzed by VELOSO *et al.* (2010). However, this did not happen, which leads to the conclusion that 'Rabo de Ovelha Tinto'

accession is misidentified and was considered a new genotype. Ampelographic analysis is in agreement with molecular data, since seven morphologic characters differ between 'Rabo de Ovelha Tinto' and 'Rabo de Anho' (Figure C). 'Esgana Cão' accession is another case of wrong denomination. The SSR profile does not match with 'Esgana Cão' of VELOSO *et al.* (2010), being considered a new genotype. Although the name 'Esgana Cão' is not well implemented to this accession, some of its characteristics, namely the fact that is a late and acid cultivar, resemble the 'Esganosos' group of cultivars (personal information).

'Sousão Galego' accession has the same SSR profile of 'Sousão Galego' accession described as a new genotype by CASTRO *et al.* (2011). Another six accessions were considered as new genotypes because none of the SSR profiles obtained corresponds to cultivar profiles that were previously described (Tabs 1 and 2).

**Chloroplast microsatellite polymorphisms:** To infer about the genetic origin of the studied autochthonous and minor grapevine accessions of Vinhos Verdes DOC Region, three polymorphic chloroplast microsatellite loci were selected (WEISING and GARDNER 1999).

Considering the accession identification performed with nuclear SSR markers, only the 34 different genotypes were analyzed with chloroplast SSR markers.

In all three ccmp loci, primer pairs yielded polymorphic products, each one with two size variants: 105 and 106 bp in ccmp3, 102 and 103 bp in ccmp 5 and 110 and 111 bp in ccmp10, showing some variability, attending to the fact that the considered cultivation area is restricted to Vinhos Verdes DOC Region. The alleles differed by 1 bp, which is consistent with the variation in the number of residues within a mononucleotide repeat.

When alleles from the three loci were combined, two different chloroplast haplotypes were detected, and designated as A (105bp-ccmp3, 103bp-ccmp5 and 110bp-

ccmp10) and D (106bp-ccmp3, 102bp-ccmp5 and 111bp-ccmp10), according to ARROYO-GARCÍA *et al.* (2002).

Chloroplast haplotype A revealed to be the most frequent, present in 33 accessions. Haplotype D was only observed in one accession, 'Carvalho 1'.

ARROYO-GARCÍA *et al.* (2006) analyzed the haplotype variation and distribution of both *Vitis vinifera* subsp. *sylvestris* and *Vitis vinifera* subsp. *sativa* genotypes from the whole area of the species' distribution and identified eight different haplotypes, designated from A to H. Their results suggest the existence of at least two important origins for the cultivated germplasm, one in the Near East and another in the western Mediterranean region. Haplotype A, is described as typical of the Iberian Peninsula wild and cultivated grapevines, which is referred as a centre of domestication of grape, and haplotype D as more frequent in other regions, as Italian Peninsula.

Other authors corroborated this idea of Iberian Peninsula as centre of domestication of grape (CUNHA *et al.* 2009, CASTRO *et al.* 2013). Thus, and considering the fact that only one accession showed the haplotype D, it is suggested that this accession had its origin outside the Vinhos Verdes DOC Region. CUNHA *et al.* (2009) also suggest that the presence of haplotype D in Portuguese cultivars can be the result of old cultivars that were brought to Portugal during the development of wine technology and due to natural crosses between wild and cultivated cultivars.

## Conclusions

This study comprised 39 accessions, which were identified and grouped into 34 different genotypes, nine of them not previously described in literature, and so, referred as new genotypes. Some new synonyms were detected, namely between the Portuguese cultivars 'Formosa' and 'Mourisco'

Table 2

List of the nine new grapevine genotypes with allele size in base pairs per each of 12 microsatellite loci analyzed

Accession	VrZAG47	VVMD5	VrZAG62	VVMD7	VVS2	VrZAG79
Amaral de Baião	157 165	228 234	185 195	237 261	130 132	243 245
Azal Sto Tirso	157 157	218 222	193 193	237 237	132 150	245 245
Bogalhal	157 165	222 234	187 195	237 261	140 156	245 249
Doçália	157 159	222 222	193 195	237 261	130 154	245 245
Esgana-cão	157 161	222 222	185 193	237 249	132 150	245 249
Loureiro Bravo	157 161	222 228	187 195	237 261	130 132	245 249
Rabo de Ovelha Tinto	157 161	228 234	187 187	237 251	150 150	245 257
Sousão Galego	155 157	222 228	185 193	237 249	132 140	245 249
Verdelho Quelho	157 165	222 234	195 195	237 269	130 132	245 249
Accession	VViv37	VVip31	VMC4f3	VVMD28	VVMD32	VViv67
Amaral de Baião	159 161	182 192	171 171	233 233	237 249	363 363
Azal Sto Tirso	161 161	182 186	177 177	233 255	237 249	360 363
Bogalhal	159 163	178 188	171 171	233 265	237 237	355 360
Doçália	161 161	190 194	165 204	231 231	249 249	353 360
Esgana-cão	157 159	178 188	171 202	233 255	237 269	363 371
Loureiro Bravo	157 161	182 188	177 177	255 265	237 237	355 363
Rabo de Ovelha Tinto	159 161	186 186	171 185	233 233	237 237	363 368
Sousão Galego	159 161	178 188	171 204	255 265	237 237	363 371
Verdelho Quelho	159 161	178 182	165 177	225 265	237 249	353 363



Table 3

Ampelographic characteristics of 12 OIV descriptors on grapevine accessions identified as new genotypes

OIV code	Azal Sto Tirso	Esgana-Cão	Sousão Galego	Verdelho Quelho	Bogalhal	Rabo de Ovelha Tinto	Doçália	Loureiro Bravo	Amaral de Baião
(001)	7	7	7	7	7	7	7	7	7
(004)	5	5	4	5	5	5	7	3	7
(016)	1	1	1	1	1	1	1	1	1
(051)	3	4	2	1	3	2	2	3	3
(067)	3	4	4	3	3	3	3	3	3
(068)	3	1	3	3	2	3	3	3	2
(070)	1	1	1	1	1	1	5	1	2
(076)	3	5	3	3	3	3	3	3	3
(079)	3	4	3	5	4	6	6	4	6
(081-2)	1	2	2	1	1	1	2	1	1
(084)	5	6	3	3	5	6	6	6	6
(087)	5	3	3	1	1	1	1	3	1

and the Spanish 'Mantúo' and 'Tinta Castañal', respectively, as well as misidentifications and wrong designations. Possible crosses and descendants involving 'Amaral' cultivar were also identified; however these results must be further investigated with a higher number of SSR loci.

Besides the genetic interest in the correct identification and preservation of these autochthonous and minor cultivars in order to prevent their extinction and maintain the biodiversity of Vinhos Verdes DOC Region, they could also be restored and introduced on the production of new and original wines.

### Acknowledgements

Joint publication of the COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding". V. FERREIRA was funded by a PhD fellowship from Fundação para a Ciência e Tecnologia (FCT) (SFRH/BD/96400/2013).

### References

- ALMADANIM, M. C.; BALEIRAS-COUTO, M. M.; PEREIRA, H. S.; CARNEIRO, L. C.; FEVEIREIRO, P.; EIRAS-DIAS, J. E.; MORAIS-CECILIO, L.; VIEGAS, W.; VELOSO, M. M.; 2007: Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal. *Vitis* **46**, 116-119.
- ARROYO-GARCÍA, R.; LEFORT, F.; DE ANDRES, M. T.; IBANAEZ, J.; BORREGO, J.; JOUVE, N.; CABELLO, F.; MARTINEZ-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.; IBANEZ, 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.; PELSUY, F.; PRIMIKIRIOS, N.; RISOVANNAYA, V.; ROUBELAKIS-ANGELAKIS, K. A.; SNOUSSI, H.; SOTIRI, P.; TAMHANKAR, S.; THIS, P.; TROSHIN, L.; MALPICA, J. M.; LEFORT, F.; MARTINEZ-ZAPATER, J. M.; 2006: Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. *Mol. Ecol.* **15**, 3707-3714.
- BRAVO P.; OLIVEIRA J. D.; 1916: *Viticultura Moderna*. Porto: Oficinas do "Commercio do Porto".
- CASTRO, I.; MARTÍN, J. P.; ORTIZ, J. M.; PINTO-CARNIDE, O.; 2011: Varietal discrimination and genetic relationships of *Vitis vinifera* L. cultivars from two major Controlled Appellation (DOC) regions in Portugal. *Sci. Hortic.* **127**, 507-514.
- CASTRO, I.; MARTIN, J. P.; ORTIZ, J. M.; MOTA, M. T.; PINTO-CARNIDE O.; 2012: The Portuguese grapevine cultivar Amaral: synonymies, homonymies and misnames. *Vitis* **51**, 61-63.
- CASTRO, I.; PINTO-CARNIDE, O.; ORTIZ, J. M.; MARTIN, J. P.; 2013: Chloroplast genome diversity in Portuguese grapevine (*Vitis vinifera* L.) cultivars. *Mol. Biotechnol.* **54**, 528-540.
- CUNHA, J.; TEIXEIRA SANTOS, M.; CARNEIRO, L. C.; FEVEIREIRO, P.; EIRAS-DIAS, J. E.; 2009: Portuguese traditional grapevine cultivars and wild vines (*Vitis vinifera* L.) share morphological and genetic traits. *Genet. Res. Crop Evol.* **56**, 975-989.
- DÍAZ-LOSADA, E.; FERNÁNDEZ, I. O.; MARTÍNEZ, F. R.; SALGADO, A. T.; CABRER, A. R.; LORENZO, S. P.; 2011a: Corbillón. In: A colección de vides da estación de viticultura e enoloxía de Galicia, 133-137. Xunta de Galicia. Consellería do Medio Rural., Santiago de Compostela.
- DÍAZ-LOSADA, E.; SALGADO, A. T.; RAMOS-CABRER, A. M.; PEREIRA-LORENZO, S.; 2011b: Determination of genetic relationships of Albariño and Loureira cultivars with the Caíño group by microsatellites. *Am. J. Enol. Vitic.* **62**, 371-375.
- GARCÍA DE LOS SALMONES, N.; 1912: Informe General de las Sesiones. In: Congreso Nacional de Viticultura. Pamplona.
- IVV; 2013: Vinhos e aguardentes de Portugal - Anuário 2012/13. Lisboa: Instituto da Vinha e do Vinho. Ministério da Agricultura e do Mar.
- MARTIN, J. P.; SANTIAGO, J. L.; PINTO-CARNIDE, O.; LEAL, F.; MARTINEZ, M. D.; 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. *Genet. Res. Crop Evol.* **53**, 1255-1261.
- MARTÍN, J. P.; ARRANZ, C.; CASTRO, I. D.; YUSTE, J.; RUBIO, J. A.; PINTO-CARNIDE, O.; ORTIZ, J. M.; 2011: Prospection and identification of grapevine varieties cultivated in north Portugal and northwest Spain. *Vitis* **50**, 29-33.
- MOTA, T.; GARRIDO, J.; CERDEIRA, A.; FERNANDES, D.; ANDRADE, I.; FRANCISCO, E.; RODRIGUES, R.; MOTA, A.; OLIVEIRA, J.; CASTRO, I.; 2013: Cultural and oenological performance of minority varieties of the Vinhos Verdes DOC region, 992-1000. In: Proc. 18<sup>th</sup> Int. Symp. GiESCO, Porto.
- OIV; 2009: 2<sup>nd</sup> Edition of the Liste des Descripteurs OIV pour les Variétés et Espèces de Vitis. 2<sup>nd</sup> ed. Paris, France: Ed. OIV.
- PINTO-CARNIDE, O.; MARTIN, J. P.; LEAL, F.; CASTRO, I.; GUEDES-PINTO, H.; ORTIZ, J. M.; 2003: Characterization of grapevine (*Vitis vinifera* L.) cultivars from northern Portugal using RADP and microsatellite markers. *Vitis* **42**, 23-25.
- SANTIAGO J. L.; BOSO S.; MARTÍN J. P.; ORTIZ J. M.; MARTÍNEZ M. C.; 2005: Characterisation and identification of grapevine cultivars (*Vitis vin-*

- ifera* L.) from northwestern Spain using microsatellite markers and ampelometric methods. *Vitis*. **44**, 67-72.
- SANTIAGO, J. L.; BOSO, S.; GAGO, P.; ALONSO-VILLAVARDE, V. ; MARTÍNEZ, M. C.; 2008: A contribution to the maintenance of grapevine diversity: The rescue of Tinta Castañal (*Vitis vinifera* L.), a variety on the edge of extinction. *Sci. Hortic.* **116**, 199-204.
- VELOSO, M. M.; ALMANDANIM, M. C.; BALEIRAS-COUTO, M.; PEREIRA, H. S.; CARNEIRO, L. C.; FEVEREIRO, P. ; EIRAS-DIAS, J.; 2010: Microsatellite database of grapevine (*Vitis vinifera* L.) cultivars used for wine production in Portugal. *Cienc. Tec. Vitivinic.* **25**, 53-61.
- WEISING, K. ; GARDNER, R. C.; 1999: A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome* **42**, 9-19.