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The Portuguese grapevine cultivar 'Amaral': synonymies, homonymies and misnames

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Summary

Within Western Europe, Portugal is the country with highest diversity of grapevine cultivars. As the Iberian Peninsula has been considered a domestication centre of grapevines, the interest on Portuguese grapevine cultivars diversity has increased in the last years.

Using twelve SSR markers scattered across twelve grape chromosomes, thirty-three accessions collected on Vinhos Verdes and Lafões Controlled Designations of Origin (DOC) and supposedly of the grapevine cultivar 'Amaral', were analysed. Names of the accessions ranged from 'Amaral' and 'Azal Tinto' to 'Sousão Galego'. Eight different genotypes were detected. The majority of accessions had the genotype attributed to 'Amaral'. However, three accessions were identified as misnaming cases and four genotypes were considered unknown, as no cultivar identification was achieved. Nevertheless, not only the misnamed cultivars, 'Vinhão', 'Melhorio' and 'Touriga Nacional', but also the four unknown genotypes, 'Azal Tinto' (AT2203 and AT2206), 'Amaral-1', 'Amaral-2' and 'Sousão Galego', share at least one allele in each of the 12 SSR loci analysed.

The accessions AT2203 and AT2206 were found as possible offspring of 'Amaral' and 'Folgasão' and the accession 'Amaral-2' is likely result of a cross between 'Amaral' and 'Pedral'.

The high number of synonym names that are attributed to 'Amaral', like 'Azal Tinto', 'Azar', 'Cainho' or 'Susão Galego' and the fact that it probably has parent-offspring relations with many cultivars, suggests that 'Amaral' is an ancient cultivar in the Northwest of the Iberian Peninsula and was a very important cultivar in the past.

Key words: autochthonous cultivars; grapevine identification; microsatellites; pedigree; synonyms; *Vitis vinifera* L.

Introduction

The number of existing grapevine (*Vitis vinifera* L.) cultivars is very high, around 5,000 based on DNA profiles,

many of them being closely related (THIS *et al.* 2006). Identification of misnames, synonymies and homonymies is an unfinished task in world viticulture. The application of fast and reliable techniques, in conjugation with ampelography, allows a correct identification of grapevine material. Microsatellite markers are one of the most useful techniques for molecular characterization of plant species. In the case of grapevines, six of those markers have been included in the descriptor list edition of the OIV (International Organisation of Vine and Wine).

In recent years, the interest on Portuguese grapevine cultivars diversity has increased since the Iberian Peninsula was considered a domestication centre of grapevines (ARROYO-GARCÍA *et al.* 2006). Portugal has a great richness in grapevine cultivars, a similar or higher number than other important wine countries.

Several homonymies, and specially synonymies, have been detected between Portugal and Spain (PINTO-CARNIDE *et al.* 2003, MARTÍN *et al.* 2006, CASTRO *et al.* 2011). 'Amaral' and the Spanish 'Caiño Bravo' represent one of these cases of synonymy in the Iberian Peninsula (MARTÍN *et al.* 2006).

Nowadays, 'Amaral' is cultivated mainly in the Northwest of Portugal, in Vinhos Verdes and Lafões Controlled Designations of Origin (DOC).

Several synonymies were attributed to 'Amaral', as 'Sousão Galego', 'Azar', 'Azal Tinto', 'Cainho Bravo', 'Cainho Miúdo' and 'Cainzinho' (MOTA and SILVA 1986). On the basis of 12 microsatellites profiling (CASTRO *et al.* 2011), 'Amaral' and 'Sousão Galego' were considered different genotypes. 'Azal Tinto' is the name most commonly attributed to the cultivar in the Vinhos Verdes Region, although it was considered, in legislation previous to 2000, different from 'Amaral'. Decree-Law No. 10/92 of the Statutes of the Vinhos Verdes Region Viticulture Commission (CVRVV) contained the two designations, 'Azal Tinto' and 'Amaral', as distinct cultivars, and currently, only the designation 'Amaral' appears in the Portuguese list of grapevine cultivars for wine production in Portugal (Ministerial order N° 428/2000).

Due to this controversy, the aim of this research was to study the relationships between supposedly close genotypes, their possible parentages, and their synonymies and homonymies.

Material and Methods

All the plant material was collected at the locations where 'Amaral' is more cultivated. A total of 33 accessions were studied, including 21 from the Vinhos Verdes DOC region: two 'Azal Tinto' from UTAD (Universidade de Trás-os-Montes e Alto Douro, at Vila Real) and EVAG (Estação Vitivinícola Amândio Galhano, at Arcos de Valdevez); three 'Amaral': 'Amaral 1' and 'Amaral 2', from EVAG and 'Amaral' from INRB (Instituto Nacional de Recursos Biológicos, at Dois Portos, Torres Vedras); one 'Sousão Galego', from EVAG; and fifteen presumed 'Azal Tinto' clones: AT0101, AT0212, AT0220, AT0701, AT0901, AT0902, AT0903, AT1303, AT1310, AT1401, AT1903, AT1906, AT2105, AT2203, AT2206, from Quinta de Soutelo, a private vineyard, located in the Basto Vinhos Verdes sub-region, that makes part of the Portuguese clonal selection program. The other 12 accessions, from the Lafões DOC region, were also presumed 'Azal Tinto' clones, from the same vineyard: AT5103, AT5105, AT5303, AT6105, AT6110, AT6111, AT6112, AT6116, AT6504, AT6711, AT6803 and AT7103.

Fresh leaves were collected in the field, frozen, and transferred to the freezer at -80 °C. Total DNA was extracted from frozen leaf material following the protocol supplied in the "NucleoSpin® Plant II Kit" (Macherey-Nagel, Düren, Germany). Quantification and analysis of the 12 microsatellite loci was carried out as previously described (CASTRO *et al.* 2011).

Results and Discussion

Amplification of the 12 microsatellite loci in the 33 accessions allowed the identification of eight different genotypes and the detection of 52 different alleles (Table).

Twenty-three out of the 33 accessions resulted in the same genotype. This genotype corresponds to the one previously published by PINTO-CARNIDE *et al.* (2003), based on the six OIV SSR loci amplification, for the 'Amaral' cultivar.

Four SSR profiles were found in the five accessions named 'Amaral 1', 'Amaral 2', 'Sousão Galego' (CASTRO *et al.* 2011, MARTÍN *et al.* 2011), AT2203 and AT2206 (these last two with the same genotype). These profiles were checked with previously published fingerprints of Portuguese cultivars and a Spanish microsatellite database, but no identification was found; consequently, they were considered as unknown or new genotypes. Nevertheless, all of them share at least one allele with 'Amaral' in each of the 12 loci amplified.

Accessions from UTAD, INRB and the clone AT0903 show the same SSR profile (Table) published for the cultivar 'Melhorio' (CASTRO *et al.* 2011). Probably the source of the error was the misnaming of the clone AT0903 that then was sent to INRB and to UTAD. Also the two accessions AT6110 and AT6112 correspond to the cultivars 'Vinhão' and 'Touriga Nacional', respectively. Both are from Oliveira de Frades municipality in Portugal. These three misnamed cultivars share morphological characteristics and

Table

Different genotypes observed in the 33 grapevine accessions studied and allele sizes in base pairs at each of 12 microsatellite loci analysed. 'Folgasão' and 'Pedral' profiles were added for discussion of possible parentage (see text for details)

Locus	Identified genotypes												Folgasão
	Melhorio	Vinhão	Touriga Nacional	unknown 1	unknown 2	unknown 3	unknown 4	Amaral	Pedral				
VVS2	140-156	130-132	140-150	130-132	132-156	132-140	132-150	132-140	150-156			130-150	
VVMD5	218-228	218-222	222-232	228-234	222-222	222-228	228-236	222-228	222-222			228-236	
VVMD7	237-261	237-261	237-237	237-261	237-261	237-249	245-261	237-249	237-261			241-245	
VVMD27	185-185	185-185	177-185	177-185	181-185	175-177	177-181	175-177	177-181			181-185	
ssrVrZAG62	187-195	187-195	187-193	185-195	185-195	185-193	195-203	185-193	185-195			193-203	
ssrVrZAG79	243-249	243-249	243-243	243-245	243-249	245-249	245-249	245-249	245-249			243-249	
VVMD28	255-265	233-255	231-265	233-233	251-265	255-265	233-255	233-255	225-251			233-255	
VVMD32	237-237	237-237	237-269	237-249	237-249	237-237	237-237	237-237	237-249			237-269	
VVv37	161-163	161-163	159-161	159-161	159-161	159-161	159-161	159-161	161-163			155-159	
VVv67	353-360	353-363	360-363	363-363	355-363	363-371	353-363	363-371	355-355			353-368	
VVlp31	178-182	178-188	182-182	182-192	178-190	178-188	178-178	178-188	188-190			174-178	
VVC4f3	171-177	171-177	177-204	171-171	177-181	171-204	177-177	171-204	171-181			177-187	
Identification of the studied cultivars	Amaral INRB; Azal Tinto UTAD; AT0903	AT6110	AT6112	Amaral 1 EVAG	Amaral 2 EVAG	Sousão Galego EVAG	AT2203; AT2206	23 remaining studied accessions					

also at least one allele in each of the 12 loci with 'Amaral', suggesting a possible parent-offspring relationship. 'Melhorio' is an old and minor cultivar that in the XIXth and early XXth centuries was restricted to the Basto sub-region in the Vinhos Verdes DOC region; 'Touriga Nacional' is designated as 'Amaral' in some Portuguese regions; 'Vinhão', a high quality red grape cultivar, is the most used in Vinhos Verdes region and recommended in all sub-regions, being the same 'Sousão' of the Douro region and the 'Sousón' of Galicia (MARTÍN *et al.* 2006; CASTRO *et al.* 2011).

'Amaral' is a synonym of the Spanish cultivar 'Caiño Bravo' (MARTÍN *et al.* 2006). Other Spanish 'Caiño' cultivars, 'Caiño Tinto', 'Caiño Blanco' and 'Caiño do Freixo', have Portuguese synonym names, 'Borraçal', 'Cainho de Moreira' and 'Espadeiro Mole', respectively (PINTO-CARNIDE *et al.* 2003, MARTÍN *et al.* 2006, CASTRO *et al.* 2011). Cultivars under the name 'Caiño' are rather frequent and have been cultivated in Spain for a long time. A very recent study (DÍAZ-LOSADA *et al.* 2011) reports the large amount of cultivars that are likely to have a parent-offspring relationship with the Spanish cultivar 'Caiño Bravo': 'Caiño Blanco' is the offspring of a cross between 'Albariño', the Portuguese 'Alvarinho', and 'Caiño Bravo'. The cultivars 'Loureira', 'Caiño Redondo', 'Caiño Longo' and 'Sousón' share a high number of alleles with 'Caiño Bravo' in 33 SSR loci analysed.

As mentioned above, the accessions with unknown genotypes share at least one allele in each locus with the true 'Amaral' (Table). A search for possible parent/offspring relations was undertaken. Results in the Table indicate that 'Amaral 2' could be the result of the cross between 'Amaral' and 'Pedral', while AT2203 and AT2206 could have originated from the cross between 'Amaral' and 'Folgasão', the Spanish 'Cagarrizo' (MARTÍN *et al.* 2011). MYLES *et al.* (2011) suggest that the considerable genetic diversity within *V. vinifera* is due to the close relationships generated by crosses among elite cultivars. Also in Portuguese cultivars some parent-offspring relationships have been reported, as 'Cercial', from 'Malvasia Fina' with 'Esgana Cão'; 'Cayetana', from 'Rabo de Ovelha' with 'Antão Vaz'; 'Lusitano', likely from 'Castelão Francês' with 'Alicante Henri Bouschet' (LOPES *et al.* 2006). Recently 'Touriga Nacional' and 'Marufo' were proposed as the parents of 'Touriga Franca' (CASTRO *et al.* 2011), and probably also of 'Tinta Barroca'.

The present study highlights the necessity to correctly identify the cultivars included in germplasm banks since misidentifications were observed. Most of the studied accessions corresponded to the true to type 'Amaral' with a

genotype defined by 12 SSR. Four unknown related genotypes as well as several misnaming and possible parentages were detected. It can be concluded that 'Amaral', frequently named as 'Azal Tinto' that also has homonymies, is closely related with other more or less ancient cultivars.

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