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Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal

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Summary

Portugal is very rich in grapevine biodiversity but nowadays only a small number of cultivars (13 black and 3 white) with defined enological characteristics are being used. However, there are still 51 cultivars with significant expression in the country which have a great potential to be used in the establishment of new vineyards. Considering the importance of identifying and understanding the genetic relatedness of the main cultivars used for wine making in Portugal, those cultivars were genotyped with the 6 SSRs loci *VVMD5, VVMD7, VVMD27, VrZag62, VrZag79* and *VVS2* that are recommended by the Office de la Vigne et du Vin for the elaboration of the worldwide database containing the alleles of all the known grapevine cultivars. A total of 49 alleles were detected with an average allele number of 8.17 per locus. The SSR profile of the 51 cultivars, at the 6 evaluated loci, indicates that all can be distinguished from each other. In addition, this study produces information on the relationship of these 51 grapevine cultivars with cultivars of other important Mediterranean wine producing regions.

K e y w o r d s : genetic relatedness; germplasm; SSR; *Vitis vinifera*.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest crops and the only Mediterranean/Western Asiatic representative of the *Vitis* genus. Its domestication created cultivars suited to a wide diversity of climates and tastes.

Portugal is very rich in grapevine biodiversity and in the XIX century 1482 different cultivar names were detected. Nowadays, many cultivars are hardly used or at extinction risk and although 341 are officially authorized for wine production only 51 are important. Modern Portuguese vineyards are mainly planted with 13 red cultivars (representing 90 % of the area) and 3 white cultivars, while in the oldest vineyards it is still possible to identify a greater number. Such an irreversible loss of diversity dangerously shrinks the genetic pool, increasing the crop vulnerability to climate changes and new pests and diseases. The *ex-situ* field collections have a crucial role in germplasm conservation. The Portuguese Grapevine Collection established in 1988, at Estação Vitivinícola Nacional (Dois Portos), where all the grapevine cultivars are preserved, has that purpose.

For cultivar identification several DNA based markers have already been used, particularly microsatellite (SSRs), which are highly informative in what concerns genetic diversity and are considered the molecular tools of choice for germplasm studies (Lopes *et al.* 1999).

The genotyping of the main Portuguese grapevine cultivars used for wine making is essential to establish their identification and contribute to management. In the present study the 6 SSRs markers established by the Office International de la Vigne et du Vin (OIV) are used to understand the genetic relatedness between the cultivars and to construct a database representing the identified alleles.

Material and Methods

Fifty-one Portuguese cultivars important for wine production (Table) were obtained from Estação Vitivinícola Nacional. DNA was isolated from young leaves according to Doyle and Doyle (1990).

SSRs recommended by OIV, VVMD5 and VVMD7 (Bowers *et al.* 1996), VVMD27 (Bowers *et al.* 1999), VrZag62 and VrZag79 (Sefc *et al.* 1999) and VVS2 (Thomas and Scott 1993), labeled with phosphoramidite linked dye, were used. PCR reactions were conducted in a Biometra Uno II thermal cycler and fragment analysis was carried out in a CEQ8000 (Beckman Coulter).

The proportion of shared alleles distance was calculated as one minus the proportion of shared alleles, between the multiple locus genotypes of two individuals using MICROSAT(http://lotka.stanford.edu/research/distance.htm). Cluster analysis was performed on shared alleles distance matrix using the UPGMA as implemented in NTSYS-pc, version 2.1 (Rohlf 2000).

Results and Discussion

Genotyping the 51 grapevine cultivars with the 6 SSR markers yielded 49 alleles, ranging from 7 (VVMD27 and VrZag62) to 11 (VVS2) alleles per locus, with a mean value of 8.17. This value is higher than previously referred for

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Table

Genetic profiles of 51 grapevine cultivars (referred by the accession number at "Colecção Ampelográfica Nacional" and the officially established name) at 6 microsatellite loci. Allele sizes are given in base pairs

		VVMD5		VVMD7		VVMD27		VrZag62		VrZag79		VVS2	
No.	Name	allele 1	allele 2	allele 1	allele 2	allele 1	allele 2	allele 1	allele 2	allele 1	allele 2	allele 1	allele 2
52003	Alfrocheiro	226	238	249	253	179	189	188	200	251	251	145	153
52007	Alvarinho	222	232	235	235	189	189	186	204	247	251	137	153
52316	Antão Vaz	234	236	245	259	181	183	204	204	247	247	147	153
52603	Aragonez	236	236	235	249	183	183	196	200	247	251	145	147
52311	Arinto	226	238	239	247	181	185	186	188	247	251	145	153
52310	Avesso	222	240	235	235	181	189	186	186	243	247	137	153
52809	Azal	226	232	235	243	181	185	194	204	247	251	153	159
52606	Baga	232	240	235	235	179	189	188	204	247	251	145	157
52803	Bastardo	238	238	235	253	175	189	188	188	245	247	145	153
52016	Bical	226	240	235	259	179	185	188	194	251	251	135	147
52402	Camarate	234	236	239	249	181	189	188	200	247	251	147	153
53106	Castelão	236	238	239	253	179	181	188	188	247	251	145	147
52410	Cerceal Branco	226	236	245	253	179	181	188	204	247	251	145	159
52207	Encruzado	226	232	235	253	183	189	194	194	247	251	151	153
52904	Espadeiro	222	226	243	259	183	189	196	204	251	251	135	153
52810	Fernão Pires	226	240	235	235	183	194	188	194	247	247	147	153
52709	Folgasão	232	240	239	243	185	189	194	204	245	251	135	153
52913	Galego Dourado	228	240	235	239	185	189	188	194	245	251	135	135
52112	Gouveio	226	238	235	239	185	189	186	188	251	251	153	159
52503	Jaen	226	236	245	253	181	189	188	194	247	251	147	153
52213	Loureiro	232	232	247	259	181	185	186	196	251	251	145	153
52512	Malvasia Fina	226	240	235	253	179	194	188	188	247	251	145	147
53013	Malvasia Rei	228	240	235	245	185	194	188	194	251	257	135	147
52301	Moreto	226	236	239	253	181	189	188	188	247	251	147	153
40705	Moscatel Graudo	228	232	245	247	179	194	186	204	247	255	135	151
41301	Moscatel Galego	226	228	235	245	179	189	186	188	251	255	135	153
52202	Negra Mole	222	240	235	235	181	181	188	196	247	259	145	159
51617	Perrum	236	240	235	239	181	185	188	188	243	247	135	147
52011	Rabo de Ovelha	222	236	235	239	181	181	188	194	247	247	139	153
52014	Rabigato	222	232	235	235	185	189	186	196	243	251	135	135
52106	Rufete	226	236	235	253	181	189	188	194	245	247	135	159
52203	Ramisco	226	238	235	259	181	185	188	196	247	251	135	159
40505	Sercial	226	238	235	249	181	185	188	194	247	259	135	153
51914	Síria	222	234	235	245	181	181	186	204	247	247	139	153
52910	Tália	226	232	245	249	179	183	194	200	245	251	135	145
52210	Terrantez	226	238	243	259	185	189	194	196	251	251	145	159
52905	Tinta Barroca	228	236	235	239	181	183	188	192	245	247	145	153
51905	Tinta Caiada	222	238	235	235	179	189	186	188	251	261	135	135
53307	Tinto Cão	232	234	235	259	181	185	186	194	247	251	135	135
51906	Tinta Miuda	226	238	235	235	179	183	186	188	251	259	141	153
52205	Touriga Franca	226	228	235	239	181	183	192	194	245	247	145	153
52206	Touriga Nacional	226	236	235	235	181	189	188	194	245	245	145	153
52710	Trajadura	226	236	235	247	181	185	186	186	247	247	145	153
53006	Trincadeira	234	238	235	245	181	185	188	204	247	251	135	153
52216	Trincadeira das Pratas	238	240	235	253	189	194	188	188	251	257	143	145
50317	Verdelho	222	232	235	253	181	189	194	196	247	251	135	153
52715	Viosinho	232	232	235	239	185	189	186	188	243	245	135	153
51902	Vinhão	222	226	235	259	189	189	188	196	245	251	135	137
52614	Vital	222	240	235	235	181	194	188	188	247	253	147	153
52807	Borraçal	232	238	235	235	181	185	194	194	247	247	135	137
52314	Fonte Cal	226	234	235	235	183	185	186	186	247	251	135	153

the Portuguese cultivars (LOPES *et al*. 1999), but lower than for the Spanish ones (IBÁÑEZ *et al*. 2003). The SSR values (Table) and the relationships between the cultivars (Figure) indicate that the 51 genotypes are distinct. 'Moscatel Graúdo', 'Aragonez' and 'Tália' cultivars, that are not of Portuguese origin, show the highest genetic distance. 'Moscatel

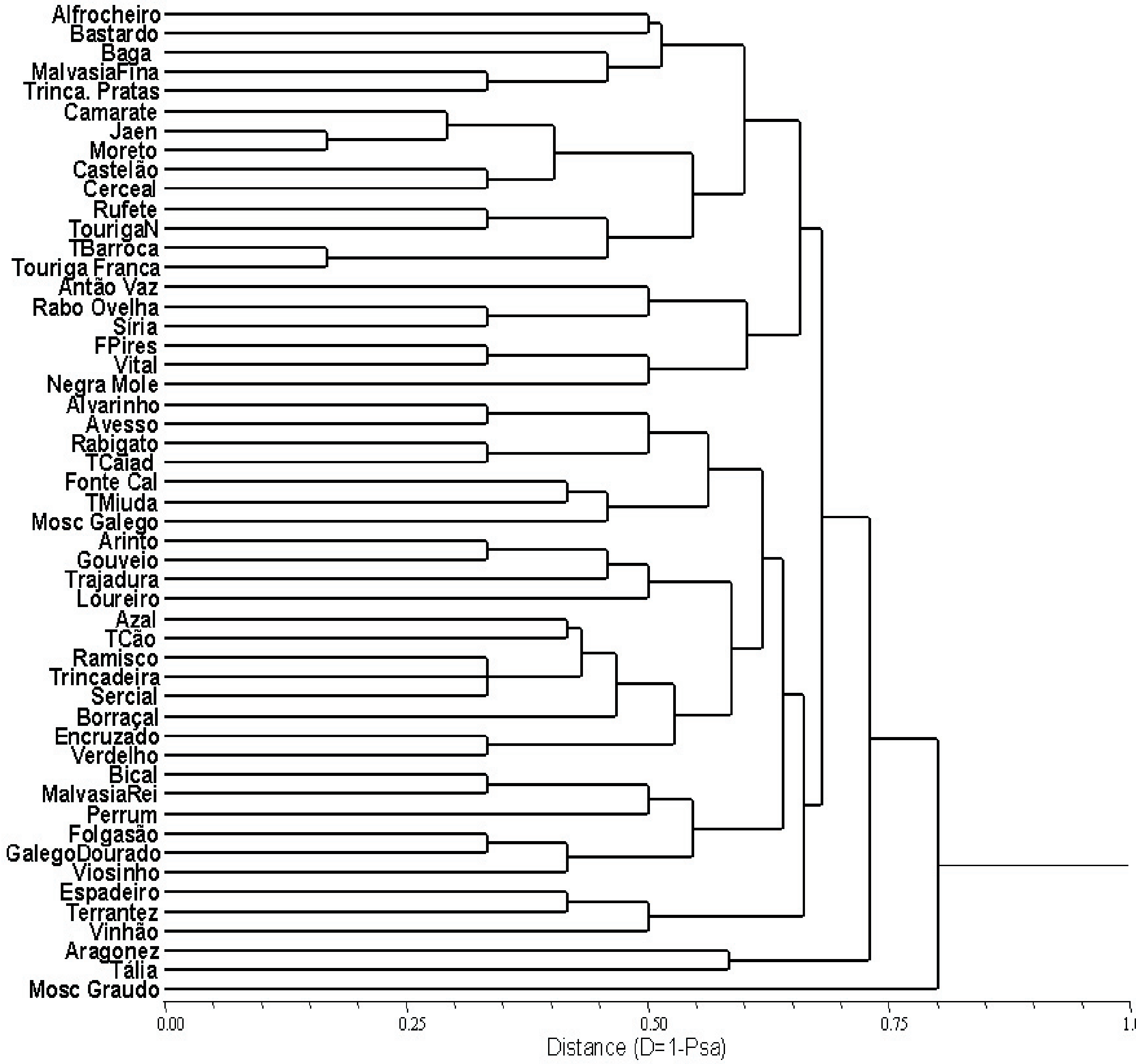

Figure: Dendrogram of 51 cultivars using D = 1- (proportion of shared alleles) as coefficient of distance and UPGMA as grouping method.

Graúdo' (synonymous to 'Moscatel de Setúbal') is the same as 'Muscat d´Alexandrie' (Lopes *et al*. 1999); 'Aragonez' (synonymous to 'Tinta Roriz') is similar to 'Tempranillo', originating from Valdepeñas (Spain) (Carneiro *et al*. 2000); 'Tália' is equivalent to 'Trebbiano Toscano' (Italy) and 'Ugni Blanc' (France) and its origine is attributed to the Tuscany region in Italy (Anonymous 1995, OIV 1996).

The pairs 'Jaen' and 'Moreto', and 'Tinta Barroca' and 'Touriga Franca' have the lowest genetic distances. Despite the genetic proximity of 'Jaen' and 'Moreto' (80 % of allele sharing) these two cultivars are considered to have distinct origins, 'Jaen' from the Bierzo region of Castilla-Léon (Spain) (Martins *et al*. 1997) and 'Moreto' from Alentejo region (Portugal). 'Tinta Barroca' and 'Touriga Franca' are both from Douro and Dão regions (Portugal).

'Sercial', 'Trincadeira' and 'Ramisco' are considered Portuguese autochthonous cultivars and are found in the oldest Portuguese vineyards. 'Ramisco' is at present neglected and only cultivated at the Colares region (near Lisbon). 'Rabo de Ovelha', 'Antão Vaz' and 'Síria' are important cultivars from Alentejo, but while 'Rabo de Ovelha' and 'Antão Vaz' are only cultivated there, 'Síria' despite its great importance in Alentejo (South Portugal) has the highest variability in the Pinhel region (North East Portugal).

'Espadeiro', 'Terrantês' and 'Vinhão' are cultivated in Portugal only in the north, but 'Vinhão' is also cultivated in the Galiza region (Spain) under the name of Souson (Martin *et al.* 2003). Its precise origin is not known.

Other Portuguese cultivars are also related to foreign ones: 'Tinta Miúda', to the Spanish Graciano (OIV 1996; Martin *et al*. 2003), introduced in Portugal at the end of the XIX century due to its resistance to oidium; 'Bastardo', to the French 'Trousseau' and the Spanish 'Merenzao' (OIV 1996), having a small genetic distance from 'Alfrocheiro', a typical Portuguese cultivar from Dão and Alentejo regions; 'Tinta Caiada', to the French 'Carcajolo' (OIV 1996), cultivated since long time in Portugal, but not important in France.

The most utilized cultivars for red wine production in Portugal are 'Touriga Nacional' and 'Touriga Franca'. The first one is a traditional cultivar of very high quality, probably from the Dão region, where the highest clonal variability is found. In contrast, 'Touriga Franca' has a low variability, suggesting that its cultivation is more recent. 'Touriga Nacional' is very close to 'Rufete', which is probably also from the Dão region.

We conclude that this study produces valuable information on the relationships between the 51 most relevant Portuguese grapevine cultivars. It should be stressed the need for studying all the others, most of which are at present greatly neglected. Indeed, all of them are of great relevance as a repository of genetic variability as they provide the raw material for future breeding programs. To protect the clonal variability of each cultivar is also of great significance. Studies that aim at the characterization of under-used cultivars and of clones of each one are being implemented in Portugal.

We also conclude that in addition to the two cultivars referred by LOPES *et al.* (1999) other Portuguese cultivars are grown in important Mediterranean wine producing regions. Their origin is not presently known.

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