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RESEARCH ARTICLE

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A comparative analysis of genetic diversity in Portuguese grape germplasm from ampelographic collections fit for quality wine production

Isaura Castro[1], Olinda Pinto-Carnide[1], Jesús M. Ortiz[2], Vanessa Ferreira[1] and Juan P. Martín[2]

[1] *Universidade de Trás-os-Montes e Alto Douro, Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas (CITAB), 5000-801 Vila Real, Portugal.* [2] *Universidad Politécnica de Madrid, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Departamento de Biotecnología-Biología Vegetal, Avda. Puerta de Hierro, 28040 Madrid, Spain*

Abstract

Grapevine cultivars diversity is vast and full of synonyms and homonyms. Up to few decades ago characterization of grapevine was based on morphological characters. In the last decades, molecular markers were developed and have been used as tools to study genetic diversity in a range of different plant species. Fifty-six Portuguese accessions representative of 'Vinhos Verdes' and 'Douro' Controlled Designations of Origin (DOC) were analysed through DNA fingerprints generated by Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR). The study aimed to compare the effectiveness of RAPD and ISSR molecular techniques in the detection of synonyms, homonyms and misnames. RAPD and ISSR analysis enabled the detection of 36 different band patterns, reducing in about 36% the initial material. Several accessions grown under different names, between and within collections, were confirmed as the same genotype, namely Gouveio/Verdelho, Sousão Douro/Vinhão and Arinto Oeste/Pedernã. Similarly, some homonyms/misnames were also identified, namely within Azal Tinto and Rabigato accessions. RAPD and ISSR markers revealed to be adequate molecular techniques for grapevine varieties fingerprinting with advantages over other molecular procedures, contributing for a good management of grapevine collections.

Additional key words: *Vitis vinifera* L.; grapevine germplasm collections; synonyms; homonyms; molecular markers; RAPDs; ISSRs.

Abbreviations used: DAMD (Directly Amplified Minisatellite DNA); DOC (Controlled Designation of Origin); EVAG (Estação Vitivinícola Amândio Galhano); ISSR (Inter Simple Sequence Repeat); MI (Marker Index); PIC (Polymorphic Information Content); RAPD (Random Amplified Polymorphic DNA); Rp (Resolving Power); SM (Simple Matching); SSR (Simple Sequence Repeat); UTAD (Universidade de Trás-os-Montes e Alto Douro).

Authors' contributions: Conceived and designed the experiments: IC, OPC, JMO, JPM. Performed the experiments: IC, JPM. Analysed the data: IC, VF, JPM. Contributed reagents/material/analysis tools: OPC, JMO, JPM. Wrote the paper: IC, OPC, JMO, VF, JPM.

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Correspondence should be addressed to Isaura Castro: icastro@utad.pt

Introduction

The first grapevine germplasm banks emerged in the late nineteenth century, after the appearance of phylloxera in Europe, from North America, in the middle of that century (Cabello *et al*., 1999). A significant loss of native plant material occurred due to the disappearance of millions of hectares produced by the attack of that insect. More recently, genetic diversity has suffered another drawback. New plantations with foreign material with low genetic variability have reinforced genetic erosion of native germplasm. Moreover, European Union incentives for restructuring and conversion, particularly in Portugal and Spain, have conducted to

the loss of hectares of old vineyards and, most probably, also autochthonous minor cultivars. Germplasm banks assume a huge importance in the preservation of local cultivars that, due to their low rentability, have a reduced area of cultivation or that, inclusive, have no longer expression in viticulture areas and their existence is restricted to collections.

The number of different cultivars held in grapevine germplasm collections around the world is estimated to be approximately 10,000 (Alleweldt & Dettweiller, 1994). Among them only few hundred are cultivated for commercial wine production (Truel *et al*., 1980). The management of these collections requires attention to avoid redundancy, to track introductions that were wrongly assigned to a cultivar and to assist clone selection (Pelsy *et al*., 2010). So, identification of the plant material is crucial and represents the first step in germplasm management (Cipriani *et al*., 2010; Laucou *et al*., 2011).

Morphology has been the most used tool in the characterization of grapevine germplasm in most of the plant collections (Boursiquot & This, 1996; Ortiz *et al*., 2004). International organisations such as the OIV (Office International de la Vigne et du Vin) or the ex-IPGRI (International Plant Genetic Resources Institute, present Bioversity International) have published useful descriptors for the ampelography and comparison studies to be carried out with the germplasm material (OIV, 2009). However, this process is carried out on adult plants; a longer period is required before the identification of accessions and often it is not conclusive on the distinction of close cultivars. As many synonyms or homonyms exist for cultivars, passport data are not always sufficient to certify identities, and errors can arise (Buhner-Zaharieva *et al*., 2010; Laucou *et al*., 2011).

In recent decades, DNA-based methodologies were implemented, enabling easier and more accurate identification of *Vitis* germplasm. Pioneer and also recent research with Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) molecular markers has been successful and widely applied to estimate genetic diversity among cultivated table and wine grapevine varieties, wild grapes and also rootstocks and to distinguish grapevine cultivars and clones, either alone (Moreno *et al*., 1995, 1998; Vidal *et al.,* 1999; Ercisli *et al*., 2008; Karatas & Ağaoğlu, 2008; Tamhankar *et al*., 2008; Jing & Wang, 2013), combined both (Herrera *et al*., 2002) and combined with other markers (Gogorcena *et al*., 1993: RAPDs and Restriction Fragment Length Polymorphisms (RFLPs); Ulanovsky *et al*., 2002 and Pinto-Carnide *et al*., 2003: RAPDs and Simple Sequence Repeats (SSRs); Seyedimoradi *et al*., 2012: ISSRs and Directly Amplified Minisatellite DNA (DAMD).

The objective of the present study was to use RAPD and ISSR molecular marker systems to evaluate the content on redundancy, synonymies and homonymies in a group of 56 Portuguese accessions from two different grapevine germplasm collections, which represent all the cultivars used for 'Vinhos Verdes' quality wines production and also many cultivars of 'Douro' and 'Porto' DOC denominations.

Material and methods

Plant material

Fifty-six Portuguese accessions were sampled in two grapevine germplasm collections from North of Portugal: (1) the ampelographic collection of the 'Vinhos Verdes Region Viticulture Commission' (CVRVV) 'Estação Vitivinícola Amândio Galhano' (EVAG) in Arcos de Valdevez, inside 'Vinhos Verdes' DOC Region, and (2) the ampelographic collection of the University 'Universidade de Trás-os-Montes e Alto Douro' (UTAD) in Vila Real, inside 'Douro' DOC Region (Table 1).

DNA isolation, RAPD and ISSR amplification

Genomic DNA was extracted from leaves using the 'NucleoSpin® Plant II Kit' (Macherey-Nagel, Düren, Germany). DNA was subsequently quantified on agarose gels and working dilutions of 10 ng/µL were made.

Sixty decamers of arbitrary sequence from OPA, OPE and OPO kits (Operon Technologies Inc., Alameda, CA, USA) and nine from the University of British Columbia Biotechnology Center (UBC) (Vancouver, Canada) were tested for the amplification of RAPD fragments. Eight RAPD primers were selected for this study, retrieving high number of amplification products, reproducible and able to be analysed without ambiguity (Table 2).

The amplification was carried out in 25 µL of reaction mixture containing 0.3 µM of the single RAPD primer, 0.2 mM of each of the four dNTPs, 2.5 mM $MgCl_2$, 0.85 U of Taq DNA polymerase in 1X manufacturer's buffer (Thermo Scientific, MA, USA), and 50 ng of template DNA. The PCR amplification was set with an initial denaturation cycle of 6 min at 94 ºC, followed by 40 cycles of 1 min at 94 ºC, 1 min at 37 ºC, 2 min at 72 ºC, and finally 10 min extension at 72 ºC.

After an initial screening using 36 ISSR primers provided in the UBC set #9, eight were selected for this study (Table 2). The amplification was carried out in 20 µL of reaction mixture containing 0.5 µM of the

Table 1. List of the 56 Portuguese grapevine accessions analysed in this study.

Accession code	Name in the collection	Germplasm collection[a]	Acession code	Name in the collection	Germplasm collection
1-AlP-U	Alfrocheiro Preto	UTAD	29-MGR-U	Moscatel Galego Roxo	UTAD
2-Alv-E	Alvarinho	EVAG	30-PaB-E	Padeiro de Basto	EVAG
3-ArB-U	Arinto Bairrada	UTAD	31-Pen-U	Pedernã	UTAD
4-ArD-U	Arinto Douro	UTAD	32-Pen-E	Pedernã	EVAG
5-ArO-U	Arinto Oeste	UTAD	33-Pel-U	Pedral	UTAD
6-Ave-U	Avesso	UTAD	34-Pel-E	Pedral	EVAG
7-Ave-E	Avesso	EVAG	35-Rab-U	Rabigato	UTAD
8-AzB-U	Azal Branco	UTAD	36-Rab-E	Rabigato	EVAG
9-AzB-E	Azal Branco	EVAG	37-Sou-U	Sousão	UTAD
10-AzT-U	Azal Tinto	UTAD	38-Sou-E	Sousão	EVAG
11-AzT-E	Azal Tinto	EVAG	39-SoD-E	Sousão Douro	EVAG
12-Bag-U	Baga	UTAD	40-SoG-E	Sousão Galego	EVAG
13-Bat-E	Batoca	EVAG	41-Tal-U	Tália	UTAD
14-Bic-E	Bical	EVAG	42-Tal-E	Tália	EVAG
15-Bor-U	Borraçal	UTAD	43-TAm-U	Tinta Amarela	UTAD
16-Bor-E	Borraçal	EVAG	44-TBo-U	Tinta Barroca	UTAD
17-Bra-U	Brancelho	UTAD	45-TCa-U	Tinta Carvalha	UTAD
18-BrA-E	Brancelho Alvarelhão	EVAG	46-TBa-U	Tinta da Barca	UTAD
19-Esp-U	Espadeiro	UTAD	47-TFr-U	Tinta Francisca	UTAD
20-Esp-E	Espadeiro	EVAG	48-TRo-U	Tinta Roriz	UTAD
21-Gou-U	Gouveio	UTAD	49-Tco-U	Tinto Cão	UTAD
22-GoD-E	Gouveio Douro	EVAG	50-ToF-U	Touriga Franca	UTAD
23-Lam-E	Lameiro	EVAG	51-ToN-U	Touriga Nacional	UTAD
24-Lou-U	Loureiro	UTAD	52-Tra-U	Trajadura	UTAD
25-Lou-E	Loureiro	EVAG	53-Tra-E	Trajadura	EVAG
26-MaF-U	Malvasia Fina	UTAD	54-Ver-U	Verdelho	UTAD
27-MaF-E	Malvasia Fina	EVAG	55-Vin-U	Vinhão	UTAD
28-MGB-U	Moscatel Galego Branco	UTAD	56-Vin-E	Vinhão	EVAG

[a] EVAG: Estação Vitivinícola Amândio Galhano (Arcos de Valdevez, Portugal); UTAD: Universidade de Trás-os-Montes e Alto Douro (Vila Real, Portugal).

single ISSR primer, 0.15 mM of each of the four dNTPs, 2 mM $MgCl_2$, 0.8 U Tth DNA polymerase in 1X manufacturer's buffer (Biotools, B&M Labs, Madrid, Spain), and 20 ng of template DNA. The PCR amplification was set with an initial denaturation cycle of 4 min at 94 ºC, followed by 35 cycles of 30 s at 94 ºC, 45 s at 50 ºC, 52 ºC or 55 ºC (see Table 2), 2 min at 72 ºC, and finally 5 min extension at 72 ºC.

The RAPD and ISSR amplifications procedure was always carried out in duplicate. Moreover, in each amplification run, 10% of samples were duplicated. Bands were considered to be reproducible when the same DNA pattern was obtained in, at least, two amplification runs.

The RAPD and ISSR products were resolved by electrophoresis on 2% agarose gels, followed by ethidium bromide staining (0.05%). The electrophoretic patterns of the PCR products were digitally recorded using the Molecular Image® Gel-Doc™ XRþ with Image Lab™ Software (BIO RAD, Hercules, CA, USA). The molecular size of fragments was estimated by reference to a DNA ladder (RAPDs - GeneRuler DNA Ladder Mix., Thermo Scientific, and ISSR - 100 bp Ladder, Pharmacia).

Data analysis

Reproducible and clearly resolved fragments in the RAPD and ISSR profiles were recorded as present (1) and absent (0). Genetic similarity matrices among accessions from RAPD and ISSR data were calculated using the simple matching (SM) similarity index (Sneath & Sokal, 1973) and employed to construct UPGMA dendrograms with the NTSYS-pc version 2.20 software package (Rohlf, 2005).

A cophenetic matrix was produced from each tree matrix to test the goodness of fit of the cluster analysis to the similarity matrix on which it was based, by comparing the two matrices using the Mantel matrix correspondence test (Mantel, 1967) in the MXCOMP program of the NTSYS-pc package.

The ability of each primer to differentiate between genotypes was assessed by calculating their resolving

Table 2. Sequences of the RAPD and ISSR primers selected for this study, and ISSR primers annealing temperatures used.

RAPD		ISSR		
Primer	Primer sequence (5'→3')	Primer	Primer sequence (5'→3') [a]	Annealing temp. (ºC)
OPA-15	TTCCGAACCC	UBC-811	$(GA)_8C$	52
OPO-03	CTGTTGCTAC	UBC-815	$(CT)_8G$	52
OPO-07	CAGCACTGAC	UBC-861	$(ACC)_6$	55
OPO-10	TCAGAGCGCC	UBC-868	$(GAA)_6$	50
OPO-19	GGTGCACGTT	UBC-881	$(GGGTG)_3$	52
UBC-523	ACAGGCAGAC	UBC-888	$DBD(CA)_7$	55
UBC-561	CATAACGACC	UBC-889	$DBD(AC)_7$	55
UBC-584	GCGGGCAGGA	UBC-890	$VHV(GT)_7$	55

[a] B for non-A, D for non-C, H for non-G, V for non-T residue.

power (Rp) according to Prevost & Wilkinson (1999). The polymorphic information content (PIC) of each marker was also calculated (Roldán-Ruiz *et al*., 2000). Marker Index (MI), defined as the product of the percentage of polymorphic bands and PIC, was used to estimate the overall utility of each marker system (Sorkheh *et al*., 2007).

Results

Polymorphism

The sixteen RAPD and ISSR primers selected (Table 2) allowed amplification of 145 fragments in the 56 Portuguese accessions studied, of which 116 (80.0%) were polymorphic (Table 3). RAPD and ISSR marker systems produced a similar average number of polymorphic bands/primer, 7.6 and 6.9, respectively. In the RAPDs analysis, all the bands generated by UBC-561 primer were polymorphic (see Fig. 1A) and the primer OPO-07 provided the highest absolute number (14) of polymorphic bands (Table 3). For ISSR, all the bands produced with the primers UBC-888 (see Fig. 1B) and UBC-889 were polymorphic and the highest number (14) was observed with UBC-888 primer (Table 3).

The PIC averages were calculated for each marker system (Table 3) and the highest mean value (0.32) was observed for RAPD markers. The highest MI (35.3) was observed in the primer UBC-561 and the highest mean MI (23.6) was observed for RAPDs (Table 3). The lowest MI values were observed in the ISSR UBC-861 (10.3) and RAPD UBC-584 (10.5) primers. The Rp reached the highest mean values for RAPDs (3.51), although the highest individual Rp value (6.46) was observed in the ISSR primer UBC-888 (Table 3).

Misnames, duplications, synonyms and homonyms

Analysis of the RAPD and ISSR marker systems individually, allowed detecting 44 and 43 distinct profiles, respectively, as can be observed in the respective dendrograms (Figs. 2 and 3). The group of 56 accessions was reduced, at 0.95 coefficient of similarity, to 37 and 36, considering the different band patterns of RAPD and ISSR marker systems, respectively (Figs. 2 and 3). The Mantel test revealed a good and significant cophenetic correlation for both markers (RAPD: $r = 0.79$; p=0.001 and ISSR: $r = 0.74$; p=0.001), evidencing that dendrograms provide a good fit for the SM similarity matrices.

The main difference in the clustering of accessions observed between the two marker systems was that, with ISSR markers (Fig. 3), the accessions Sousão (UTAD), Sousão (EVAG), Sousão Douro (EVAG), Vinhão (UTAD) and Vinhão (EVAG) grouped in the same cluster with a similarity higher than 0.95, while RAPD markers (Fig. 2) allowed the separation of these five accessions in two clusters, one with Sousão (UTAD) and Sousão (EVAG) and the other with the remaining three samples, Sousão Douro (EVAG), Vinhão (UTAD) and Vinhão (EVAG).

Some synonymies previously identified and registered at the Vitis International Variety Catalogue (www.vivc.de) were observed in both collections according to the RAPD and ISSR markers analysed, namely [Vinhão/Sousão Douro], [Gouveio/Gouveio Douro/Verdelho] and [Arinto Oeste/Pedernã] (Table 4; Figs. 2 and 3; Fig. S1 [suppl.]) with similarity levels higher than 0.95.

Given the RAPD and ISSR profiles obtained, several supposed misnames and/or homonymies were detected, namely between the groups of accessions with different designation [Arinto Bairrada (UTAD)/Baga

Table 3. Results of the observed genetic diversity in the 56 Portuguese grapevine accessions studied.

	TB	PB (%)	Rp	PIC	MI
RAPDs					
OPA-15	10	8 (80.0)	3.71	0.33	26.8
OPO-03	10	6 (60.0)	3.75	0.41	24.5
OPO-07	16	14 (87.5)	5.07	0.27	23.7
OPO-10	9	7 (77.8)	3.68	0.36	27.6
OPO-19	11	8 (72.7)	3.82	0.32	23.4
UBC-523	11	7 (63.6)	3.04	0.26	16.6
UBC-561	8	8 (100.0)	3.93	0.35	35.3
UBC-584	7	3 (42.9)	1.07	0.25	10.5
Total	82	61 (74.4)			
Mean	10.3	7.6	3.51	0.32	23.6
ISSRs					
UBC-811	3	2 (66.7)	1.04	0.31	20.9
UBC-815	4	3 (75.0)	1.89	0.41	30.7
UBC-861	4	2 (50.0)	0.46	0.21	10.3
UBC-868	4	3 (75.0)	1.11	0.21	15.9
UBC-881	8	6 (75.0)	2.04	0.25	18.7
UBC-888	14	14 (100.0)	6.46	0.31	30.8
UBC-889	12	12 (100.0)	5.18	0.29	29.2
UBC-890	14	13 (92.9)	4.29	0.22	20.5
Total	63	55 (87.3)			
Mean	7.9	6.9	2.81	0.28	22.1

TB, total of bands; PB, polymorphic bands; Rp, resolving power; PIC, polymorphic information content; MI, marker index.

Table 4. Groups of accessions with identical patterns in either RAPD, ISSR or in both, at levels of similarity both 1.0, either 1.0 and 0.95 and both 0.95

ISSR 1.0 similarity level RAPD 1.0 similarity level	ISSR 0.95 similarity level RAPD 1.0 similarity level	ISSR 1.0 similarity level RAPD 0.95 similarity level	ISSR 0.95 similarity level RAPD 0.95 similarity level
6-Avesso (UTAD) 7-Avesso (EVAG)	3-Arinto Bairrada (UTAD) 12-Baga (UTAD)	8-Azal Branco (UTAD) 9-Azal Branco (EVAG)	5-Arinto Oeste (UTAD) 31-Pedernã (UTAD) 32-Pedernã (EVAG)
15-Borraçal (UTAD) 16-Borraçal (EVAG)	17-Brancelho (UTAD) 18-Brancelho Alvarelhão (EVAG)	19-Espadeiro (UTAD) 20-Espadeiro (EVAG)	27-Malvasia Fina (EVAG) 14-Bical (EVAG)
21-Gouveio (UTAD) 22-Gouveio Douro (EVAG) 54-Verdelho (UTAD)	41-Tália (UTAD) 42-Tália (EVAG)	31-Pedernã (UTAD) 32-Pedernã (EVAG)	39-Sousão Douro (EVAG) 55-Vinhão (UTAD) 56-Vinhão (EVAG)
24-Loureiro (UTAD) 25-Loureiro (EVAG)		55-Vinhão (UTAD) 56-Vinhão (EVAG)	
28-Moscatel Galego Branco (UTAD) 29-Moscatel Galego Roxo (UTAD)			
33-Pedral (UTAD) 34-Pedral (EVAG)			
37-Sousão (UTAD) 38-Sousão (EVAG)			
52-Trajadura (UTAD) 53-Trajadura (EVAG)			

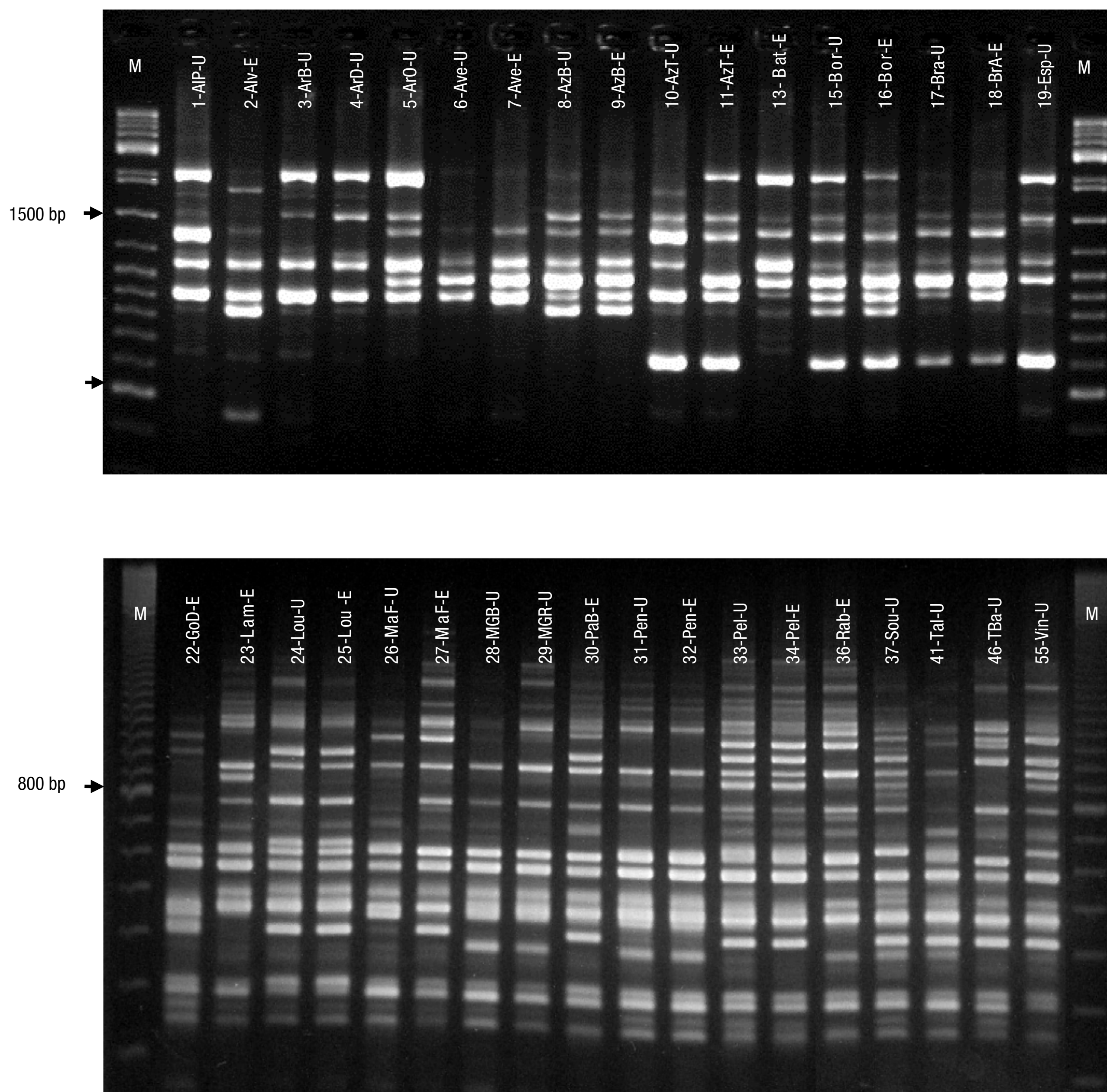

Figure 1. Profiles obtained on 2% agarose gels for **(A)** 17 accessions using the UBC-561 RAPD primer, M – GeneRuler DNA Ladder Mix (Thermo Scientific), and **(B)** 18 accessions using the UBC-888 ISSR primer, M – 100 bp Ladder (Pharmacia). Accessions code in Table 1.

(UTAD)] and [Malvasia Fina (EVAG)/Bical (EVAG)], given their clustering at high level of similarity (Table 4, Figs. 2 and 3). On the contrary, in the accessions Azal Tinto and Rabigato, sampled both in EVAG and UTAD, with the same designation, different band patterns were detected suggesting misnaming or homonymy (Figs. 2 and 3).

The marker systems used showed some potential in the clonal discrimination. Inside the groups of accessions [Azal Branco (EVAG)/Azal Branco (UTAD)]; [Espadeiro (EVAG)/Espadeiro (UTAD)]; [Pedernã (EVAG)/Pedernã (UTAD)]; [Tália (EVAG)/Tália (UTAD)] and [Vinhão (EVAG)/Vinhão (UTAD)], similarity was slightly below 1.0 in one of the markers systems (Table 4); therefore, intracultivar variability was observed with these markers, however, the confirmation is still under discussion.

Discussion

In the present study, RAPD and ISSR markers were used to analyse the variability in 56 Portuguese accessions of *V. vinifera* cultivars. In several cases, for ac-

cessions with the same designation, different samples collected in two different Portuguese grapevine germplasm collections were analysed (Table 1).

The conservation of band profiles demonstrates the reproducibility of both techniques used in the study. RAPD and ISSR molecular techniques accumulate several advantages, namely, small amount of DNA required, facility of technical procedure, no fluorescence labeling, no need for information on template DNAs or the synthesis of specific primers and simultaneous amplification of several loci. So, their use is adequate to screen high number of samples in order to detect duplicated material, suspicious of erroneous identifications or synonyms and homonyms.

Few RAPD and ISSR primers (see Table 2) were needed to generate highly diagnostic and reproducible fingerprint. The RAPD primer OPO-07 and the ISSR primers UBC-888, UBC-889 and UBC-890 used in this study, revealed a high capacity for grapevine cultivars discrimination (Table 3) given the high number of total and polymorphic bands, which is in agreement with the results of Zietkiewicz *et al*. (1994) and Moreno *et al*. (1998) for the ISSR amplifications with 5' three-anchored primers (UBC-888, UBC-889 and UBC-890).

Using RAPD and ISSR markers 37 and 36 different molecular profiles were obtained, respectively, within the 56 accessions analysed, at 0.95 coefficient of similarity (Figs. 2 and 3). Likewise, Lopes *et al*. (1999), studying 49 supposed different cultivars from the Portuguese National Ampelographic Collection and from the Grapevine Collection of Terceira (Azores islands) through microsatellite loci amplification, detected some synonym cases and only 36 different SSR profiles were found. Similarly, Laucou *et al*. (2011) analysed 4,370 accessions of the INRA grape repository at Vassal with 20 microsatellite markers and only found 2,836 SSR single profiles. Cipriani *et al*. (2010) analysed 1,005 grapevine accessions from CRA-VIT of Conegliano collection by amplifying 34 microsatellite loci, identified 200 groups of synonyms and only 745 unique genotypes and Buhner-Zaharieva *et al*. (2010) in germplasm analysed from the Movera Grapevine Germplasm Bank in Aragón, Spain, found in 36 autochthonous accessions only 24 SSR profiles, besides 33 misnamed accessions.

Combination of molecular and morphological characterization methodologies has led to a good management of grapevine genetic resources (Ortiz *et al*., 2004; Balda *et al*., 2014; Ferreira *et al*., 2015; Maul *et al*., 2015). In a characterization of *V. vinifera* L. accessions from the Spanish gene bank at Alcalá de Henares, Ortiz *et al.* (2004) using morphological descriptors, isoenzymes and microsatellites, reduced the number of different accessions from 621 to 177, which represents less than 30% of the initial number. In the scope of the COST Action FA1003, in 997 accessions of Eastern European cultivars analysed through ampelography and nine microsatellite markers amplification, only 659 unique profiles/cultivars were found (Maul *et al*., 2015).

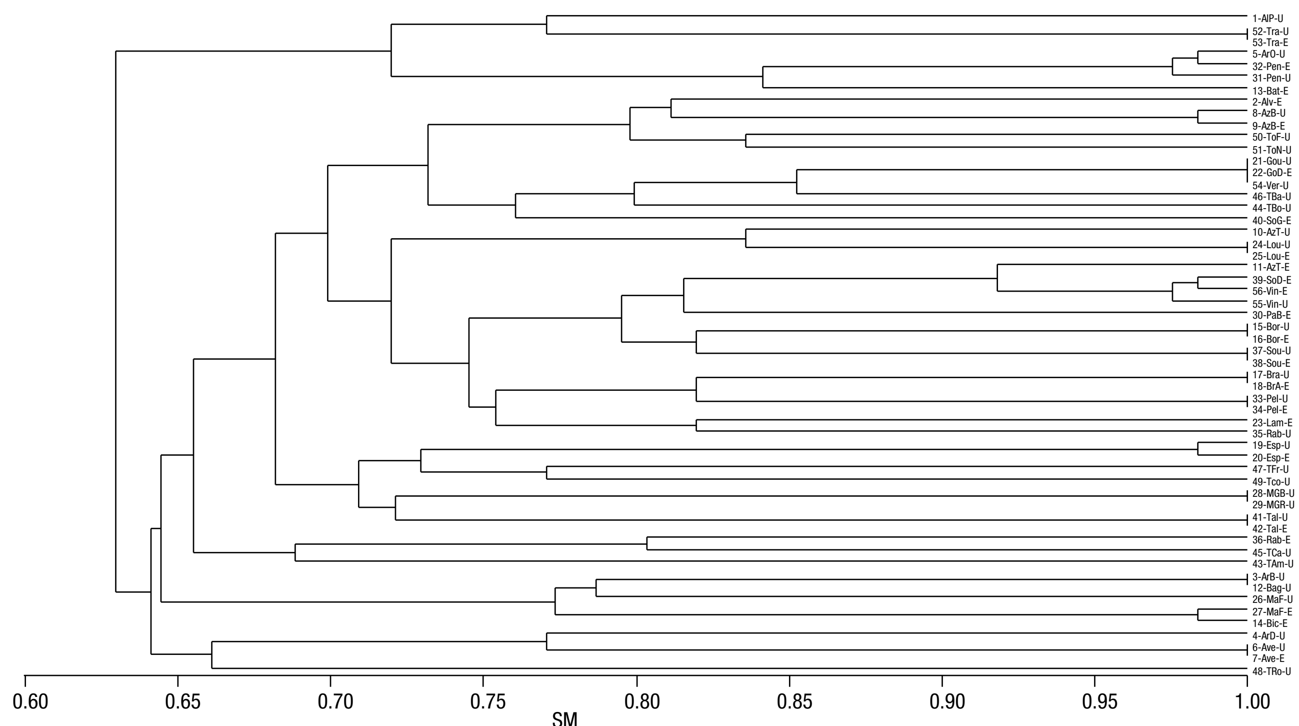

Figure 2. Dendrogram of 56 Portuguese grapevine accessions studied obtained using UPGMA cluster analysis of RAPD marker data. Accessions code in Table 1. SM: simple matching coefficient of similarity.

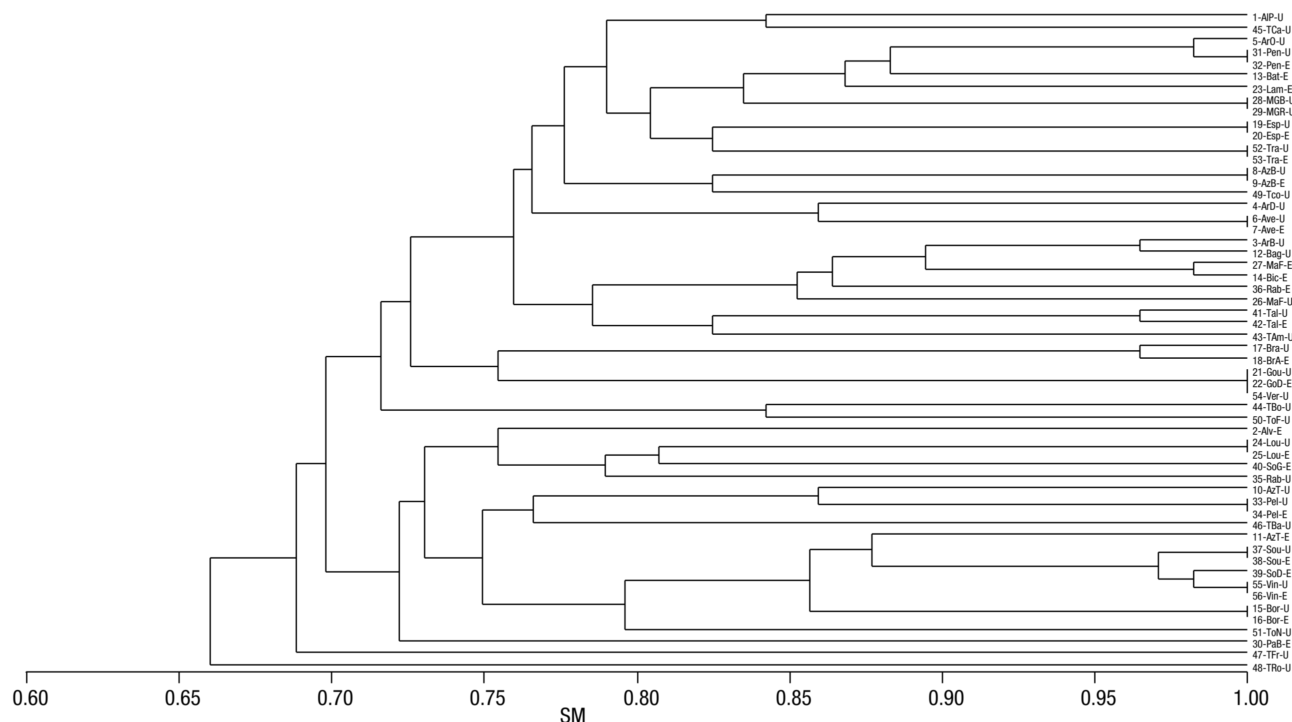

Figure 3. Dendrogram of 56 Portuguese grapevine accessions studied obtained using UPGMA cluster analysis of ISSR marker data. Accessions code in Table 1. SM: simple matching coefficient of similarity.

Of the 56 accessions that were studied, Moscatel Galego Branco and Moscatel Galego Roxo are considered to be berry skin-colour mutants and so, ampelographically well distinguishable. In addition to the RAPD and ISSR markers used in the present study, as expected, also nuclear microsatellites amplification performed in these cultivars failed to distinguish this pair of cultivars (Ferreira *et al.*, 2016). Other berry skin–colour mutants have been reported, namely colour variants of Muscat of Alexandria cultivar, as sharing the same molecular fingerprint at trueness-to-type established loci, and differing in specific genes related to anthocyanin biosynthesis (De Lorenzis *et al.*, 2015).

Results highlight the genetic proximity between Sousão and Vinhão accessions. Sousão is the prime name of a cultivar grown especially in 'Vinhos Verdes' Region whose cultivar designation was modified to Sezão in the last review of the 'Portuguese List of Varieties fit for Wine Production' (DR, 2012). Vinhão has been reported as the synonym of the Spanish cultivar Sousón (Martín *et al.*, 2006). However, a focus of confusion exists in Douro Region, where, frequently, is given the name Sousão to the cultivar Vinhão. Though, the observed separated RAPD clusters for Sousão and Vinhão groups are correct. Nevertheless, the fact that these accessions have a miscellaneous of names between the two RAPD clusters and that they cluster together in the ISSR marker analysis, suggest that Sousão and Vinhão accessions are genetically close.

Also Gouveio Douro (EVAG), Gouveio (UTAD) and Verdelho (UTAD) clustered together in both molecular markers systems (Table 4; Figs. 2 and 3; Fig. S1 [suppl.]). In this case, the accessions Verdelho and Gouveio Douro are most probably the cultivar officially designated Gouveio; in some localities of 'Douro' Region, Gouveio is designated as Verdelho (Pereira & Sousa 1990). Care must be taken to not confuse with the variety Verdelho, code PRT50317 (DR, 2012), a different variety, much used for 'Madeira' wine production with cultivation restricted to the island.

Pedernã is the local designation in 'Vinhos Verdes' DOC Region for the official cultivar named Arinto (Mota & Silva, 1986), and so the accessions Arinto Oeste (UTAD)/Pedernã (EVAG)/Pedernã (UTAD) have an identical profile (Table 4; Figs. 2 and 3; Fig. S1 [suppl.]). The designation of UTAD's accession as 'Arinto Oeste' is surely due to the great importance of Arinto in the Bucelas DOC Region, located in west of Portugal, near Lisbon, where it is also known as 'Arinto de Bucelas'.

RAPD and ISSR band patterns suggest a few cases of misidentifications between and within collections. The accession Arinto Bairrada (UTAD) revealed the band pattern of Baga (UTAD). This coincidence can be explained considering that Baga, in 'Bairrada' Region, is frequently designated Tinto Bairrada. The accessions Malvasia Fina (EVAG) and Bical (EVAG) were found to be very close but different from the

accession Malvasia Fina (UTAD) that clustered at a distant similarity level. That suggests misidentification of Malvasia Fina in EVAG collection. Also, the accessions Azal Tinto (UTAD)/Azal Tinto (EVAG) and Rabigato (UTAD)/Rabigato (EVAG) revealed band patterns quite different (Figs. 2 and 3). Azal Tinto is the synonym of Amaral (Caño Bravo in Spain, Martín *et al*., 2006) and is mentioned as having several descendants (Castro *et al*., 2012; Lacombe *et al*., 2013). One of the Azal Tinto accessions may be in fact other genotype, eventually its relative. The different Rabigato profiles can be explained considering that there is a cultivar in 'Vinhos Verdes' DOC Region, Rabo de Ovelha, which is commonly designated Rabigato (Mota & Silva 1986).

A major obstacle to good management of grapevine germplasm banks is the persistence of synonyms and homonyms up to the current days in the viticulture worldwide. Some germplasm banks accumulate the responsibility of multiplication and commercialization of grapevine material. Efforts are being done and have to proceed in order to find an official name for propagation and distribution. The management of several grapevine collections has been carried out with the assistance of molecular markers, namely in Iberian Peninsula (Lopes *et al*., 1999; Ibáñez *et al*., 2003; Martín *et al*., 2003, 2006; Santiago *et al*., 2007; Santana *et al*., 2008; Veloso *et al*., 2010; Castro *et al*., 2011; Balda *et al*., 2014; Alifragkis *et al*., 2015) with great relevance in the correction of identification mistakes. In specific, RAPD and ISSR PCR-based fingerprinting are informative for estimating the extent of genetic diversity and patterns of genetic relationships among grape accessions in germplasm holdings (Dhanorkar e*t al*., 2005; Ercisli *et al*., 2008; Karatas & Ağaoğlu, 2008; Zeinali *et al*., 2012).

Results evidence the necessity of grapevine material characterization in ampelographic collections, complementing morphological descriptors with molecular markers for duplicates and synonyms detection.

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