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The use of microsatellites for germplasm management in a Portuguese grapevine collection

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Abstract To initiate the characterization of the Portuguese grapevine genepool, we have genotyped 49 Portuguese grapevine cultivars at 11 microsatellite loci. The markers proved to be informative in the Portuguese cultivars, with expected heterozygosity ranging from 0.67 to 0.84. At most loci, an excess of heterozygous individuals was observed, while the deficiency of heterozygotes at 1 locus (VVMD6) indicated the presence of null alleles. On the basis of the microsatellite allele data several previously assumed synonyms were verified: (1) 'Fernão Pires'='Maria Gomes', (2) 'Moscatel de Setúbal'='Muscat of Alexandria', (3) 'Boal Cachudo'='Boal da Madeira'='Malvasia Fina', (4) 'Síria'='Crato Branco'='Roupeiro' and (5) 'Periquita'='Castelão Francês'='João de Santarém'='Trincadeira'. Although the three varieties 'Verdelho da Madeira', 'Verdelho dos Açores', and 'Verdelho roxo' are regarded by the Lista Nacional de Sinónimos as distinct cultivars, they displayed identical SSR profiles at 17 loci and appear to represent types of 1 single cultivar. The genetic profiles of all 49 cultivars were searched for possible parent-offspring groups. The data obtained revealed the descendence of 'Boal Ratinho' from 'Malvasia Fina' and 'Síria'.

Key words *Vitis* · Portuguese grapevines · Germplasm management · Genotyping · Microsatellites

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Introduction

Grapevine is the most important perennial crop worldwide. The world's collections of grape plant material are estimated to contain about 5000–15.000 cultivars. One of the problems in the management of these germplasm collections is the use of synonymous and homonymous cultivar designations. The identification and comparison of plant material by ampelographic methods is afflicted by misinterpretations; DNA-based markers provide a more reliable alternative for cultivar identification (Bourquin et al. 1993; Tschammer and Zyprian 1994; Silvestroni et al. 1997; Moreno et al. 1998). Due to their codominance, high information content and easy scoring, microsatellites, or simple-sequence repeats (SSRs), are ideal molecular markers. A number of SSR primers have been developed for *Vitis* by different groups (Thomas and Scott 1993; Bowers et al. 1996; Sefc et al. 1999), and the usefulness of SSR markers for grapevine genotyping, cultivar identification, parentage studies, and detection of synonyms has been shown (Cipriani et al. 1994; Thomas et al. 1994; Botta et al. 1995; Bowers et al. 1996; Bowers and Meredith 1997; Sefc et al. 1997, 1998a,b). The ability to unambiguously distinguish between cultivars and to clarify synonyms and homonyms will be of major importance solving problems concerning the management of grapevine germplasm. The reliability of the SSR technique allows a comparison of results obtained by different groups and will help in assimilating the data into a common database.

The National List of Grapevine Synonyms of Portugal (Lista Nacional de Sinónimos das Castas do Instituto da Vinha e do Vinho) contains about 450 varieties, most of which are specific to Portugal. It is very common in Por-

tugal that identical genotypes are named differently in separate regions, as in the case of the cultivar known as 'Malvasia Fina' in the region Douro, the same variety is called 'Boal Cachudo', when grown in Ribatejo and 'Assario Branco' or 'Arinto' in the region of Dão.

In the study presented here our purpose was to evaluate the usefulness of SSR markers in germplasm collections, where many synonyms have to be expected. Hence, a set of 49 Portuguese grapevine cultivars has been genotyped at 11 microsatellite loci in order to initiate a characterization of the Portuguese grapevine genepool by molecular means. Several cases of suspected synonyms have been investigated and the genotypes were searched for possible pedigrees among the cultivars.

Material and methods

Plant material was obtained from the Grapevine Collection of the Estação Vitivinícola Nacional in Dois Portos and from the Grapevine Collection of Serviços Agrícolas in Biscoitos, Terceira.

DNA was extracted from leaves following the method described by Thomas et al. (1993). Forty nine grapevine cultivars grown on the Portuguese mainland and in the Azores (Table 1) were genotyped at the following 11 SSR loci: VVS2 (Thomas and Scott 1993), VVMD5, VVMD6, VVMD7 (Bowers et al. 1996), ssrVrZAG21, ssrVrZAG47, ssrVrZAG62, ssrVrZAG64, ssrVrZAG67, ssrVrZAG79, and ssrVrZAG83 (Sefc et al. 1999). Polymerase chain reaction (PCR) analysis was performed in 20 µl of a mixture containing 50 ng DNA, 1 µ*M* of each primer, 100 µ*M* of each dNTP, 1 U *Taq* DNA polymerase in reaction buffer (10 m*M* TRIS pH 8.8, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.1% Triton×100). One primer of each pair was labeled with the fluorescent Cy-5 dye to enable detection of the fragments in the ALFexpress automated sequencing system (Pharmacia Biotech, Vienna).

A two-step PCR protocol (Smith et al. 1995) was chosen for amplification: 95°C for 5 min, 10 cycles of 50°C for 15 s, 94°C for 15 s, followed by 23 cycles of 50°C for 15 s and 89°C for 15 s.

Table 1 Grapevine cultivars investigated in this study

Alfrocheiro	Maria Gomes
Alvarelhão	Moscatel de Setúbal
Alvarinho	Negra Mole
Antão Vaz	Periquita
Arinto de Bucelas	Rabo de Ovelha
Arinto no Douro	Ramisco
Arinto dos Açores	Roupeiro
Avesso	Rufete
Azal Branco	Saborinho
Baga	Síria
Bical	Terrantez
Boal Cachudo	Tinto Cão
Boal da Madeira	Tinta Miúda
Boal Ratinho	Tinta Roriz
Camarate	Touriga Nacional
Castelão Francês	Trajadura
Crato Branco	Trincadeira
Esgana Cão	Trincadeira Preta
Fernão Pires do Oeste	Verdelho dos Açores
F. P. do Vinho Verde	Verdelho da Madeira
F. P. do Bêco no Ribatejo	Verdelho roxo
Jaen	Vinhão
João de Santarém	Viosinho
Loureiro	Vital
Malvasia Fina	

PCR products were analyzed on a sequencing gel (6% acrylamide, 1×TBE buffer, 7 *M* urea) in an automated sequencing apparatus (ALFexpress, Pharmacia Biotech, Vienna). Fragment lengths were estimated using internal size standards (Cy5-labeled PCR products of pUC19) by Fragment Manager software (Pharmacia).

The search for identical genotypes and for parent-offspring groups as well as calculations of expected heterozygosity (He), probability of identity (PI), and probability of null alleles (r) (Brookfield 1996) were carried out using a software written by Horst W. Wagner (Institut für Allgemeine Physik, Vienna). The following formulas were applied:

$$He = 1 - \Sigma p_i^2$$
$$PI = \Sigma p_i^4 + \Sigma\Sigma(2p_i p_j)^2$$
$$r = (He - Ho)/(1 + He)$$

p_i and p_j are the frequencies of allele i and j, respectively, He and Ho are the expected and observed heterozygosity, respectively. Deviations of observed heterozygosity values from Hardy-Weinberg expectations were analyzed using the program GENEPOP. Genetic distances between cultivars were calculated in MICROSAT (Minch 1997) as (1-proportion shared alleles) and a phenogram was drawn using the UPMA algorithm in PHILIP (Felsenstein 1989) and the program TREEVIEW (Page 1996).

Results and discussion

Characterization of 11 SSR markers in Portuguese grapevine cultivars

Forty-nine grapevine cultivars grown on the Portuguese mainland and the Azores (Table 1) were genotyped at 11 SSR loci. Although these markers have already been evaluated in a set of cultivars (Thomas et al. 1994; Bowers et al. 1996; Sefc et al. 1998a, 1999), we first assessed their information content in the Portuguese grapevine population. Among the 49 cultivars analyzed, 36 different SSR profiles were detected. Only the distinguishable genotypes were included in the characterization of the SSR markers. The number of alleles per locus ranged from five alleles at VVMD6 to nine alleles at VVS2 (Table 2). The mean number of alleles in the Portuguese cultivars was seven alleles per locus, which is in agreement with previous results (Sefc et al. 1999).

The alleles of the markers used in this study were found to be evenly distributed in the Portuguese grapevines, resulting in low probabilities for identical genotypes (PI: 0.08–0.18). However, at ssrVrZAG79, the frequencies of two alleles (246 bp and 250 bp long) added up to 80%, while the remaining 20% was shared by six alleles. The uneven distribution of allele frequencies was reflected by a relatively high probability of obtaining identical profiles at ssrVrZAG79 (PI=0.27) in spite of the high number of eight alleles (Table 2). The same number of alleles, but the highest information content was provided by marker VVMD5 with a PI value of 0.08. Here, the frequency of the most common allele (224 bp) was only 23%. The probability of obtaining identical genotypes using all 11 markers is 7.4×10^{-10}, corresponding to the statistical potential to distinguish about 50 000 unrelated cultivars.

A second estimate of the information content of markers is the expected percentage of heterozygous indi-

Table 2 Genetic parameters of 11 SSR markers in 49 Portuguese cultivars. The table shows the sample size, the number of alleles detected in the cultivars, the probability of finding identical genotypes for each locus and for all loci combined, expected and observed heterozygosity, as well as the probability of null alleles at each locus

Locus	Sample size	Number of alleles	Probability of identity (PI)	Expected heterozygosity	Observed heterozygosity	Probability of null alleles
VVS 2	36	9	0.11	0.80	0.97	–0.095
VVMD 5	36	8	0.08	0.84	0.92	–0.039
VVMD 6	35	5	0.18	0.76	0.60	0.089
VVMD 7	36	8	0.15	0.71	0.72	–0.009
ssrVrZAG 21	36	6	0.15	0.79	0.89	–0.056
ssrVrZAG 47	36	6	0.15	0.77	0.81	–0.018
ssrVrZAG 62	35	6	0.16	0.76	0.83	–0.042
ssrVrZAG 64	36	8	0.11	0.81	0.97	–0.087
ssrVrZAG 67	36	7	0.16	0.77	0.86	–0.050
ssrVrZAG 79	36	8	0.27	0.67	0.72	–0.033
ssrVrZAG 83	36	6	0.18	0.76	0.75	0.007
	PI for all loci:	7.4×10^{-10}				

viduals. The marker evaluation using the values for expected heterozygosity is in good agreement with results obtained by PI values, indicating VVMD5 as the most informative and ssrVrZAG79 as the least informative marker for the Portuguese cultivars (Table 2).

At 9 of the 11 loci, the observed level of heterozygosity was higher than the expected values (Table 2). Significant excess of the number of heterozygous individuals over Hardy-Weinberg expectations was observed at loci VVS2 and ssrVrZAG 64 (P_{VVS2}=0.0036; $P_{ssrVrZAG64}$=0.0044). An excess of heterozygous individuals may be caused by selection for yield and quality. Grapevines are known to be very sensitive to inbreeding depression and higher performance is achieved by heterozygous individuals.

In contrast, the observed heterozygosity was significantly lower than the expected values at locus VVMD6 (P = 0.0177). A possible explanation is the assumption of null alleles at this locus. While other factors as mating constraints due to breeding activity might also be responsible for an excess of homozygous cultivars, such influences would be reflected in all of the loci investigated. Using the formula of Brookfield (1996), the probability of null alleles at VVMD6 is 8.9% (Table 2). In comparison, this value is 0.7% at ssrVrZAG83, and is negative for the other loci. Bowers et al. (1996) have discussed the presence of null alleles at VVMD6 because of the failure of amplification of this locus in a table grape variety, which might be a homozygous null genotype.

Investigation of synonymous cultivars

The Lista Nacional de Sinónimos das Castas (available from the Instituto da Vinha e do Vinho at http://www.ivv.pt/vinho/sinonimia.html) and the Catálogo das Castas (Eiras Dias et al. 1988) were used to compare assumptions on synonymous and homonymous cultivar designations with the data we obtained by SSR analysis of Portuguese grapevine cultivars at 11 loci. The cultivars investigated in our study are listed in Table 1, and some of the Portuguese vine growing regions relevant to this study are shown in Fig. 1.

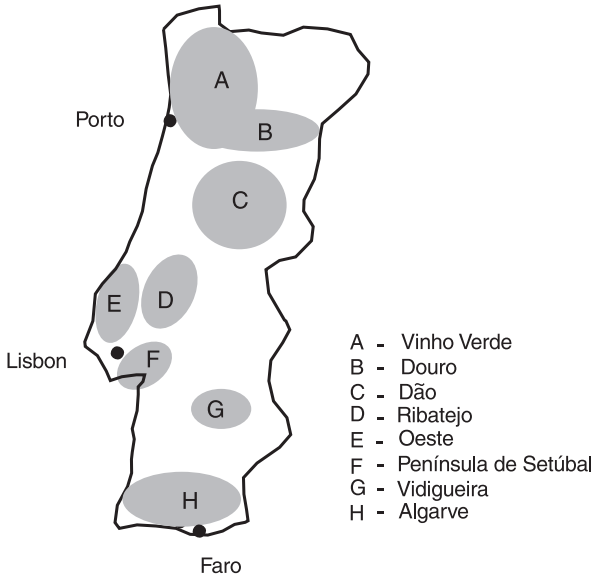

Fig. 1 Map showing Portuguese vine growing regions referred to in the text

The most important white wine cultivar of Portugal is ‘Fernão Pires’, also known as ‘Maria Gomes’. In order to investigate whether the name ‘Fernão Pires’ is used for distinct genotypes in the different regions, or whether one single genotype is addressed by this name, we analyzed ‘Fernão Pires’ from three geographic origins (Oeste, Vinho Verde and Ribatejo) and one individual of ‘Maria Gomes’. Identical genotypes were detected in all four plants (Table 3), indicating that the names ‘Fernão Pires’ and ‘Maria Gomes’ unambiguously refer to one single genotype.

Among the Portuguese red wine cultivars, ‘Periquita’ is probably the most valued one. In the two lists mentioned above, three synonyms are described for ‘Periquita’: ‘Castelão Francês’, ‘João Santarém’, and ‘Trincadeira’. SSR analyses of the 4 cultivars confirmed their synonymous state (Table 3).

‘Síria’, ‘Crato Branco’ and ‘Roupeiro’ have also been defined as synonyms, which was confirmed by our microsatellite data (Table 6).

Table 3 Confirmation of synonymy of the cultivar groups 'Fernão Pires' (from three geographic origins)/'Maria Gomes', 'Periquita'/'Castelão Francês'/'João Santarém'/'Trincadeira', and 'Moscatel de Setúbal'/'Muscat of Alexandria' by comparison of 11 SSR loci. Allele sizes are given in basepairs

[a] The – symbol indicates that the cultivar might be either homozygous or heterozygous with a null allele.

Cultivar Locus	Fernão Pires (Bêco) F.P. (Vinho Verde) F.P. (Oeste Maria Gomes	Periquita Castelão Francês João Santarém Trincadeira	Moscatel de Setúbal Muscat of Alexandria
VVS 2	144:150	142:144	132:148
VVMD 5	224:238	234:236	226:230
VVMD 6	207:209	206:-[a]	189:209
VVMD 7	236:236	240:254	246:248
ssrVrZAG 21	202:204	200:202	190:206
ssrVrZAG 47	161:172	157:159	157:172
ssrVrZAG 62	187:193	187:187	185:203
ssrVrZAG 64	141:159	139:141	139:141
ssrVrZAG 67	126:139	126:126	126:126
ssrVrZAG 79	246:246	246:250	246:254
ssrVrZAG 83	188:190	188:190	188:188

Table 4 Genotypes of cvs 'Verdelho dos Açores', 'Verdelho da Madeira', and 'Verdelho roxo' are compared at 17 SSR loci. Allele sizes are given in basepairs

Cultivar Locus	Verdelho da Madeira Verdelho dos Açores Verdelho roxo
VVS 1	161:180
VVS 2	132:150
VVS 3	212:218
VVS 29	168:168
VVMD 5	220:230
VVMD 6	199:–[a]
VVMD 7	236:254
VVMD 8	138:138
ssrVrZAG 21	204:206
ssrVrZAG 25	225:245
ssrVrZAG 47	159:167
ssrVrZAG 62	193:195
ssrVrZAG 64	159:163
ssrVrZAG 67	132:139
ssrVrZAG 79	246:250
ssrVrZAG 83	188:194
ssrVrZAG 112	234:240

[a] The – symbol indicates that the cultivar might be either homozygous or heterozygous with a null allele

Table 5 Genotypes of cvs 'Arinto de Bucelas', 'Arinto no Douro', 'Arinto dos Açores', and 'Esgana Cão'. Numbers represent sizes of alleles in basepairs

Cultivar	Arinto de Bucelas	Arinto no Douro	Arinto dos Açores	Esgana Cão
Locus				
VVS 2	142:150	134:150	132:150	132:150
VVMD 5	224:236	236:238	224:236	224:236
VVMD 6	207:–[a]	199:207	207:209	207:209
VVMD 7	240:248	236:254	236:250	236:250
ssrVrZAG 21	200:206	206:206	190:200	190:200
ssrVrZAG 47	159:163	159:167	159:163	159:163
ssrVrZAG 62	185:187	187:193	187:193	187:193
ssrVrZAG 64	137:159	137:159	137:159	137:159
ssrVrZAG 67	139:151	132:151	132:139	132:139
ssrVrZAG 79	246:250	246:250	246:258	246:258
ssrVrZAG 83	194:194	188:190	190:200	190:200

[a] The – symbol indicates that the cultivar might be either homozygous or heterozygous with a null allele

'Moscatel de Setúbal', which is used for the production of savory white wine and table grapes, is commonly considered to be one of the synonyms for 'Muscat of Alexandria' (Eiras Dias et al. 1988; Ambrosi et al. 1994). This assumption could be confirmed by our SSR data (Table 3). At the end of the 18th century, 'Muscat of Alexandria' was already widespread in the Mediterranean region, and several synonyms were known. The name 'Muscat of Alexandria' points to the origin of this cultivar from Africa, while the Portuguese designation 'Moscatel de Setúbal' indicates its cultivation on the Setúbal peninsula (Fig. 1).

'Verdelho Branco' (or 'Verdelho da Madeira') is the most important white wine variety in Madeira, where it is used for the production of the famous Madeira wine. Two other Verdelho types are cultivated in the Azores: 'Verdelho roxo' with red berries and 'Verdelho branco dos Açores' with white berries. The Lista Nacional de Sinónimos regards the three Verdelho types as distinct cultivars. However, the 3 cultivars displayed identical SSR profiles at all 11 loci used in this study. In order to strengthen the results, 6 additional loci were used [VVS1, VVS3, VVS29 (Thomas and Scott 1993), VVMD8 (Bowers et al. 1996), ssrVrZAG25, and ssrVrZAG112 (Sefc et al. 1999)] (Table 4). Furthermore, two individuals of 'Verdelho dos Açores' were analyzed to verify the clonal state of this cultivar. All individuals showed an identical genotype. We may thus conclude that the Verdelhos grown in Madeira and in the Azores represent one single cultivar and that 'Verdelho Roxo' is a berry color mutant, as has been suggested earlier (Lopes et al. in press). Berry color types, which are indistinguishable by microsatellite analysis, have already been reported for several cultivars (Sefc et al. 1998a).

SSR profiles of Arinto types from three wine growing regions, Arinto from Bucelas, Arinto from Douro, and Arinto from the Azores (Island of Terceira) have been compared. 'Arinto de Bucelas' and 'Arinto no Douro' are listed as different cultivars in the Lista Nacional de Sinónimos, and this classification could be confirmed by

Table 6 Genotypes of the synonymous cultivar groups 'Malvasia Fina'/'Boal Cachudo'/'Boal da Madeira' and 'Síria'/'Crato Branco'/'Roupeiro' have been compared at 11 SSR loci (see Material and methods). The accordance of the genotypes of 'Malvasia Fina' and 'Síria' with their offspring 'Boal Ratinho' has been proven at 23 loci. Allele sizes are given in basepairs. At locus VVMD6, 'Malvasia Fina' and 'Boal Ratinho' are assumed to be heterozygous with a null allele. Locus ssrVrZAG 12 is known to contain null alleles, and the – symbol indicates that the cultivar might be either homozygous or heterozygous with a null allele

Cultivar Locus	Malvasia Fina Boal Cachudo Boal da Madeira	Boal Ratinho	Síria Roupeiro Crato Branco
VVS 1	180:189	180:180	180:180
VVS 2	142:144	136:144	136:150
VVS 3	212:218	212:218	218:218
VVS 4	166:166	166:166	166:174
VVS 29	168:170	168:176	168:176
VVMD 5	224:238	220:238	220:232
VVMD 6	206:–[a]	209:–[a]	199:209
VVMD 7	236:254	246:254	236:246
VVMD 8	144:183	144:175	140:175
ssrVrZAG 7	155:157	108:155	108:155
ssrVrZAG 12	158:–[a]	158:–[a]	153:158
ssrVrZAG 15	165:165	165:165	165:167
ssrVrZAG 21	200:204	202:204	200:202
ssrVrZAG 25	236:245	225:236	225:236
ssrVrZAG 29	112:112	112:112	112:112
ssrVrZAG 30	149:149	147:149	147:149
ssrVrZAG 47	157:172	159:172	159:159
ssrVrZAG 62	187:187	187:203	185:203
ssrVrZAG 64	141:143	141:159	139:159
ssrVrZAG 67	126:151	126:139	126:139
ssrVrZAG 79	246:250	246:246	246:246
ssrVrZAG 83	190:194	188:190	188:190
ssrVrZAG 112	229:240	229:240	229:240

[a] The "–" symbol indicates that the cultivar might be either homozygous or heterozygous with a null allele

their characteristic and unique microsatellite profiles (Table 5). The Arinto from Terceira, however, shares its genotype with cv 'Esgana Cão' from the Portuguese mainland (Table 5), which is also known as 'Sercial' in Madeira. Currently, we are not able to tell, whether all of the Arinto types grown in Terceira and on the other Azorean islands was identical to 'Esgana Cão' or whether the Arinto cultivars from the Azores are a heterogeneous group consisting of several genotypes. Ampelographic observations suggest that the Arinto from Terceira is different from the Arinto grown on Pico, another Azorean island. More samples of Azorean Arinto have to be genotyped to answer this question.

In the Catálogo das Castas, 'Boal Cachudo' is mentioned to be identical to 'Malvasia Fina' from Douro, while the Lista Nacional de Sinónimos distinguishes between 'Boal Cachudo' and 'Boal Cachudo do Ribatejo' (not included in our study), indicating only the latter as the synonym for 'Malvasia Fina'. In our study, 'Boal Cachudo' and 'Malvasia Fina' showed a common SSR profile. Surprisingly, an identical profile was found in 'Boal da Madeira' (Table 6). These results are in agreement with previous isozyme analysis (Bernardes Carneiro 1997).

The origin of 'Boal Ratinho'

The genotypes of the investigated cultivars were searched for possible parent-offspring groups. While five families were detected based on the data of the 11 SSR markers, four of these relationships were rejected after adding of 12 more loci [VVS1, VVS3, VVS4, VVS29 (Thomas and Scott 1993), VVMD8 (Bowers et al. 1996), ssrVrZAG7, ssrVrZAG12, ssrVrZAG15, ssrVrZAG25, ssrVrZAG29, ssrVrZAG30, ssrVrZAG112 (Sefc et al. 1999)]. Data obtained from 23 markers indicated, that 'Boal Ratinho' originates from a cross between Malvasia Fina (with the synonyms 'Boal da Madeira' and 'Boal Cachudo') and 'Síria' (synonyms: 'Roupeiro', 'Crato Branco') (Table 6). In addition to the match of the allele sizes, the parentage is supported by the fact that three rare alleles are inherited by 'Boal Ratinho': 136 bp (VVS2), 246 bp (VVMD7), and 172 bp (ssrVrZAG47). In order to ensure the correct identification of the 3 cultivars we genotyped additional independent individuals.

Since only one allele (209 bp) was detected at VVMD6 in 'Boal Ratinho', we assume that a null allele has been transmitted from 'Malvasia Fina' to its offspring. The frequency of null alleles at VVMD6 in the Portuguese cultivars was shown to be 9% (see above). The inheritance of non-amplifying alleles has already been observed in crosses leading to the grapevine cultivars 'Ruby Seedless', 'Marroo Seedless' and 'Sultana Moscata' (Thomas et al. 1994) and to 'Blauburger' and 'Müller Thurgau' (Sefc et al. 1997).

'Síria' is an old Portuguese cultivar found all over the country; it was first mentioned in 1531 and is thought to originate from the Beira Interior region (E. Eiras Dias personal communication). 'Malvasia Fina' has likewise been grown in Portugal for a long period and is found in the regions Dão, Douro, Ribatejo, Algarve, and Alentejo (Ambrosi et al. 1994). Most probably the cross between 'Malvasia Fina' and 'Síria' occurred spontaneously, made possible both by their common geographic distribution and by their long cultivation history.

Similarity analysis of cultivars

In order to illustrate the population structure among the Portuguese cultivars, we constructed a phenogram, which clusters the cultivars according to their similarities calculated as proportion of shared alleles (Fig. 2). The average similarity of all cultivars is 36% shared alleles, which is close to the 40% average similarity observed for mid-European cultivars (Sefc et al. 1998a), even though in the present study more polymorphic markers were used. The phenogram indicates 3 cultivars as standing outside of the population: 'Moscatel de Setúbal', 'Antão Vaz' and 'Tinta Roriz'.

'Moscatel de Setúbal' is assumed to have been introduced to Portugal from Egypt, which explains its high genetic distance from the other Portuguese cultivars.

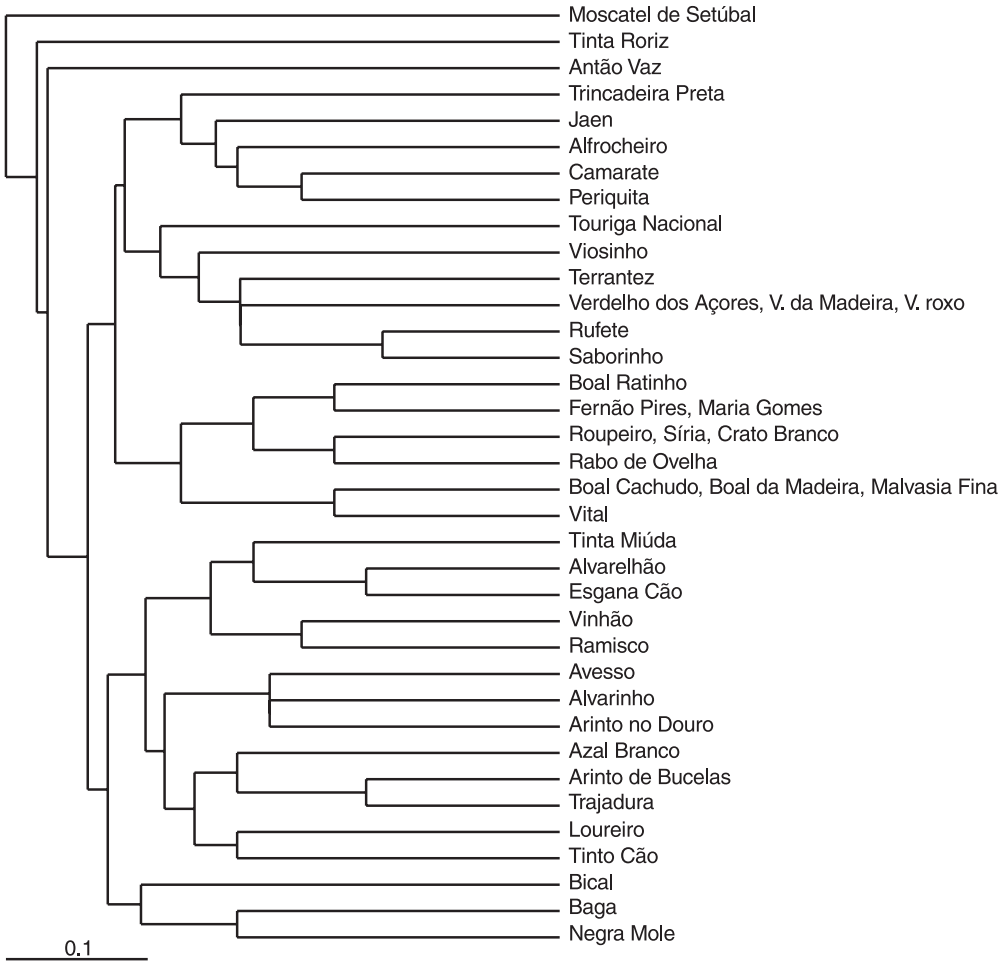

Fig. 2 Phenogram of Portuguese grapevine cultivars

Until recently, 'Antão Vaz' has been grown mainly in the area of Vidigueira do Alentejo. Only recently, due to progress in fermentation technology which allows the typical aroma of the grapes to be preserved, has the relative importance of this cultivar increased (E. Eiras Dias, personal communication). Therefore, 'Antão Vaz' may not have mingled much with the other Portuguese grapevines, which would explain its high genetic distance from the remaining cultivars.

'Tinta Roriz', the third cultivar standing apart from the Portuguese group, is a synonym for 'Tempranillo', the Spanish cultivar used for the production of the famous Rioja wines. Though widely grown in Portugal nowadays, the cultivar originates most probably from the Spanish Rioja region, which may be the cause for its genotypical separation from the Portuguese cultivars.

The average similarities between these 3 cultivars and all others are 22% for 'Moscatel de Setúbal', 26% for 'Tinta Roriz' and 27% for 'Antão Vaz'. In contrast, the highest average similarity between a cultivar and the group is 42% for 'Arinto de Bucelas'. In pairwise comparisons, the greatest distances (only 9% similarity) were found between 'Alvarelhão' and 'Tinta Roriz', and between 'Antão Vaz' and 'Bical' as well as between 'Antão Vaz' and 'Vinhão'. The highest pairwise similarities were detected between 'Rufete' and 'Saborinho' (75%), 'Alvarelhão' and 'Esgana Cão' (73%), and 'Trajadura' and 'Arinto de Bucelas' (73%).

'Boal Ratinho' and its parents 'Malvasia Fina' and 'Síria' are found in one cluster, with 23% of the alleles shared between the two parents, and similarities of 58% and 74% between 'Boal Ratinho' – 'Malvasia Fina' and 'Boal Ratinho' – 'Síria', respectively.

Conclusion

We have shown that germplasm collections which contain a high number of synonyms can reliably be characterized by microsatellite analysis. The 11 microsatellite markers chosen for this study are informative in the Portuguese grapevine population. Investigations of putatively synonymous and homonymous cultivars have resulted in the confirmation of several previous assumptions, while the rejection of some conjectures shows that it is still worthwhile to revise hypotheses.

The 49 cultivars investigated in this study are only a small part of the ensemble of cultivars grown in Portugal, but already within this limited section, a pedigree has been identified. The genetic characterization of a

larger number of cultivars will not only help to verify more homonymous and synonymous cultivars but will also reveal the genetic relationship between the Portuguese cultivars.

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