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Determination of relationships among autochthonous grapevine varieties (*Vitis vinifera* L.) in the Northwest of the Iberian Peninsula by using microsatellite markers

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Abstract

Fifty six grapevine varieties traditionally grown in the Northwest region of the Iberian Peninsula were analysed for six microsatellite loci, in order to determine the relationships among them as well as the plant material that should be collected and preserved in germplasm banks. Previous morphological and molecular results were taken into account for assessment of the existing synonymies among accessions from different European countries. Percent distribution of the main alleles was calculated. Multivariate analysis was carried out and similarities among the studied material were described and commented.

Introduction

The grapevine growing area of the northwest of the Iberian Peninsula mainly includes three regions in Spain (Galicia, Asturias and the Arribes del Duero), and two other regions in Portugal (Douro and Vinhos Verdes). Historically the relationship among these regions has been more or less intense. As a consequence, the presently grown varieties may include synonymies as well as genetically related material. In a recent study of varieties from northern Portugal using RAPD and microsatellite markers (Pinto-Carnide et al. 2003), several synonymies with Spanish varieties were detected.

The use of molecular markers is a useful methodology to complete the ampelography to detect similarities, and to define genetic relationships among grapevine varieties (Bowers et al. 1993; Sefc et al. 1999).

The present study includes a broad representation of the autochthonous grapevine cultivars of the region, and attemps to confirm the existing synonymies to establish the genetic relationships among the plant material in order to preserve the maximum amount of genetic variability for breeding and commercial purposes.

Material and methods

Plant material

A total of 272 accessions of grapevine sampled at different locations in the northwest of Spain

(Misión Biológica de Galicia (CSIC), Asturias and the west of Castilla y León, and the north of Portugal (Arcos de Valdevez (EVAG) and Vila Real (UTAD)) were studied. They were complemented with other accessions sampled at the BGVCAM germplasm bank, located at Alcalá de Henares (Madrid, Spain) (Cabello 1995), originally coming from the same geographical areas.

In all cases, sampling consisted of young fresh leaves that were collected in the field and kept at −80 °C until analysed.

STMS analysis

DNA extraction and amplification were carried out by using the MasterPure^{TM} Plant Leaf Purification Kit (Epicentre Technologies, Madison Wis.), and the following six STMS loci were used: VVS2 (Thomas and Scott 1993), VVMD5 and VVMD7 (Bowers et al. 1996), and ssrVrZAG47, ssrVrZAG62 and ssrVrZAG79 (Sefc et al. 1999), under the conditions detailed in a previous work (Martín et al. 2003). Polymorphism of the amplified products was detected in an automated DNA sequencer ABI PRISM model 310 (PE Applied Biosystems). As a result of the analysis, genotypes for each variety were obtained for the studied loci.

Table 1 summarises the number of accessions analysed for each variety, varying from a minimum of two in less than 30% of the varieties, up to a maximum of 14 in the variety Tempranillo (Tinta Roriz).

Multivariate analysis was carried out by converting the data matrix with the results of the analysis (Table 2) in a double state (0,1) matrix based on the presence or absence of each specific allele for each studied variety. From this matrix, and using the NTSYS software (Rohlf 1998), a dendrogram was obtained by applying the UPGMA method with the Dice's coefficient (Dice 1945).

Results and discussion

The studied accessions represent a broad sampling of the existing variability in the studied region of the Iberian peninsula. Some of the material has been previously studied by using RAPD markers (Leal et al. 2003) and ampelography (Santiago et al. 2003).

Some of the material is already included either in the germplasm bank at El Encín in Spain or at the collection at Arcos de Valdevez in Portugal. The rest of the varieties (Table 1), are only located in the field where they were sampled.

All the varieties with a number of accessions lower than 6 in Table 1 are considered as minor varieties and most of them have a marked risk of extinction since only isolated plantations with a reduced number of plants have been detected.

Each group of accessions of the same variety (Table 1) gave identical allele sizes (Table 2) although intravarietal variability may exist. Based on previous ampelographic and molecular studies (Rodríguez-Torres et al. 2000; Pinto-Carnide et al. 2003; Santiago et al. 2003) synonymies for about half of the studied varieties are listed in the above mentioned Table. In all cases these synonymies were confirmed by the STMS results of the present work. Twenty six varieties have at least one synonymy and 16 of them have one varietal name in Spanish and one synonymy in Portuguese, which corresponds to the geographical proximity of both growing regions. Some of the synonymies were previously mentioned in the bibliography (O.I.V. 1996) and in a previous article by the group (Pinto-Carnide et al. 2003), while some others which have not been previously mentioned, mostly refer to minor local varieties and are less well known to viticulturists.

The allele sizes at each of the six analysed loci are shown in Table 2. For those varieties previously analysed in a germplasm bank located in Spain (Martín et al. 2003), the genotypes are coincident, except in some misnames, namely Albarín Blanco and Caíño Bravo. Three alleles (231, 245 and 249) for locus VVMD7 and one allele (259) for locus ssrVrZAG79 are only present in Moscatel de Grano Menudo, Terrantez, Treixadura and Saborinho, respectively.

The number of alleles for each locus varies from 7 to 10, somewhat lower than in the previously mentioned work (Martín et al. 2003), that was expected by the more concentrated origin of the samples and their lower number.

With respect to homozygosity, Table 3 shows the obtained percentages, that oscillate between 12′5 and 25%, values that are similar to the ones

Table 1. Plant material included in the study.

No. acc.[a]	Code	Origin[b]	Color	Variety and synonymies
4	AGUDB	E	B	AGUDELO; CHENIN BLANC
4	ALBAB	G	B	ALBARÍN BLANCO
12	ALBAN	E,A	N	ALBARÍN NEGRO; BRUÑAL; ALFROCHEIRO PRETO
4	ALVAB	E,G,R	B	ALBARIÑO; ALVARINHO
5	ALBIB	E,G	B	ALBILLO MAYOR; TURRUNTÉS
4	BICAB	E,G	B	BICAL; BORRADO DAS MOSCAS
8	BRANN	E,R	N	BRANCELLAO; BRANCELHO
4	CAIBB	G,R	B	CAIÑO BLANCO; CAINHO DE MOREIRA
5	CAIBN	G	N	CAIÑO BRAVO
2	CAILN	G	N	CAIÑO LONGO
7	CAITN	E,G,R	N	CAIÑO TINTO; BORRAÇAL
2	CARRN	E	N	CARRASQUÍN
2	CASTN	G	N	CASTAÑAL
2	CERCB	V	B	CERCIAL
6	DONAB	E,G,A	B	DOÑA BLANCA; MOZA FRESCA; CIGÜENTE
6	ESVAN	E	N	ESPADEIRO
5	FERNB	E,V	B	FERNAO PIRES
6	FERRN	E,G	N	FERRÓN
4	GAJON	A	N	GAJO ARROBA
10	GODEB	E,V	B	GODELLO; GOUVEIO
9	JUANN	E,A	N	JUAN GARCÍA; MOURATÓN
2	LADOB	G	B	LADO
4	LOURB	E	B	LOUREIRA; LOUREIRO BLANCO
8	MANDN	E,A	N	MANDÓN
6	MENCN	E,V	N	MENCÍA; JAEN
9	MEREN	E,A	N	MERENZAO
5	MORRN	E	N	MORRASTEL-BOUSCHET; GARNACHO
10	MOSCB	E,A,V	B	MOSCATEL DE GRANO MENUDO; MUSCAT Á PETIT GRAINS; MOSCATEL GALEGO
6	NEGRN	E,V	N	MOLLAR CANO; NEGRA MOLE
4	PEDRN	E,G	N	PEDROL
4	PETIN	E	N	PETIT BOUSCHET; NEGRÓN DE ALDÁN
3	PUESN	E,A,V	N	PUESTO MAYOR; SABORINHO
7	RABIB	A,V	B	RABIGATO; PUESTA EN CRUZ
4	RABOB	E,V	B	RABO DE OVELHA
2	RABON	V	N	RABO DE OVELHA TINTA
4	RUFEN	E,A,V	N	RUFETE; TINTA PINHEIRA
6	SAVAB	E,G	B	SAVAGNIN BLANC; TRAMINER (G)
6	SOUSN	E,G,V	N	SOUSÓN; SOUSAO; VINHAO
14	TEMVN	E,V	N	TEMPRANILLO; TINTA RORIZ
2	TERRB	E	B	TERRANTEZ
3	TAMAN	V	N	TINTA AMARELA; TRINCADEIRA PRETA
2	TBARN	V	N	TINTA BARROCA
2	TCAON	V	N	TINTO CÃO
2	TCARN	V	N	TINTA CARVALHA
2	TFEMN	E	N	TINTA FEMIA DE ALDÁN
2	TFRAN	V	N	TINTA FRANCISCA
6	TJERN	A	N	TINTA JEROMO
2	TBRAN	V	N	TINTO DO BRAGAO
2	TGALN	E	N	TINTO GALLEGO
9	TORRB	E,G,V	B	TORRONTÉS; BOAL CACHUDO
2	TOFRN	V	N	TOURIGA FRANCESA
3	TONAN	V	N	TOURIGA NACIONAL
3	TOFEN	V	N	TOURIGO FEMEA
3	TREIB	E,G,V	B	TREIXADURA; TRAJADURA
2	VERDN	A	N	VERDEJO COLORADO
10	VIOZB	V	B	VIOSINHO

[a] Number of studied accessions.
[b] origin of the sample (A = Arribes del Duero, Castilla y León, Spain; E = Encín, Madrid, Spain; G = Galicia/Asturias, Spain; R = Arcos de Valdevez, Portugal; V = Vila Real, Portugal).

Table 2. Allele sizes (in base pairs) at each STMS loci analyzed, in the 56 studied varieties.

Code[a]	STMS loci VVS2		VVMD5		VVMD7		ssrVrZAG47		ssrVrZAG62		ssrVrZAG79	
AGUDB	130	150	224	228	237	255	151	165	187	193	245	249
ALBAB	130	150	218	234	237	255	157	165	185	193	243	245
ALBAN	140	150	222	234	251	255	155	165	187	199	249	249
ALVAB	132	150	218	228	237	237	165	165	185	203	245	249
ALBIB	140	142	228	232	237	251	159	171	185	199	249	255
BICAB	130	142	222	236	237	261	155	161	187	193	249	249
BRANN	130	150	218	222	237	237	161	165	187	193	249	257
CAIBB	140	150	218	222	237	261	157	165	195	203	245	249
CAIBN	132	140	222	228	237	261	157	165	193	195	243	245
CAILN	140	150	222	222	237	261	157	157	185	195	245	245
CAITN	130	132	228	234	237	237	157	161	193	193	245	245
CARRN	140	150	222	234	237	255	155	165	187	193	249	249
CASTN	132	154	222	222	261	261	157	165	193	195	245	257
CERCB	140	156	222	232	247	255	155	157	187	203	245	249
DONAB	134	150	218	230	237	247	157	157	185	203	245	245
ESVAN	136	150	232	234	237	241	151	165	187	187	243	245
FERNB	142	150	222	236	237	237	159	171	187	193	245	245
FERRN	130	154	232	236	237	247	165	165	193	199	249	249
GAJON	134	140	230	234	247	251	157	165	199	203	245	249
GODEB	150	156	222	234	237	241	161	165	185	187	249	249
JUANN	134	150	230	234	247	255	157	165	187	203	245	249
LADOB	130	150	228	232	241	261	157	165	185	187	243	245
LOURB	140	150	228	228	249	261	157	161	185	195	245	249
MANDN	140	150	222	236	237	237	159	171	185	187	255	257
MENCN	142	150	222	232	247	255	157	165	187	193	245	249
MEREN	140	150	234	234	237	255	151	165	187	187	243	245
MORRN	136	150	222	230	237	241	157	159	187	187	241	257
MOSCB	130	130	224	232	**231**[b]	247	155	171	185	195	249	253
NEGRN	140	142	218	236	237	237	157	157	187	195	245	257
VEDRN	150	156	222	222	237	261	157	161	185	195	245	249
VETIN	130	150	230	234	237	241	157	165	187	195	241	243
VUESN	130	150	222	234	237	255	157	165	187	193	243	**259**[b]
RABIB	130	130	218	228	237	237	161	165	185	195	241	249
RABOB	134	150	218	232	237	241	157	157	187	193	245	245
RABON	132	156	218	222	237	261	157	161	185	195	245	249
RUFEN	130	156	222	232	237	255	157	165	187	193	243	245
SAVAB	150	150	228	234	241	255	165	165	187	193	243	249
SOUSN	130	132	218	222	237	261	165	165	187	195	243	249
TEMVN	140	142	232	232	237	251	159	159	195	199	245	249
TERRB	140	148	222	234	**245**[b]	261	161	165	193	195	249	249
TAMAN	130	150	230	234	237	247	157	161	187	203	245	249
TBARN	140	150	224	232	237	241	157	159	187	191	243	245
TCAON	130	130	228	230	237	261	157	161	185	193	245	249
TCARN	142	150	228	232	247	261	157	165	193	203	245	249
TFEMN	130	142	222	232	237	237	159	165	187	195	241	245
TFRAN	130	130	234	236	237	237	161	165	185	187	241	245
TJERN	134	140	222	232	247	251	155	157	199	203	245	249
TBRAN	140	140	222	228	237	237	157	159	187	187	243	255
TGALN	130	130	222	234	241	247	155	171	187	203	253	255
TORRB	140	142	222	236	237	255	155	171	187	187	245	249
TOFRN	140	150	222	224	237	241	157	159	191	193	243	245
TONAN	140	150	222	232	237	237	157	165	187	193	243	243
TOFEN	140	140	232	236	237	255	155	165	187	193	243	249
TREIB	140	150	222	232	237	**249**[b]	157	161	185	185	245	245
VERDN	140	148	228	234	241	241	155	165	187	203	247	247
VIOZB	130	150	228	228	237	241	161	165	185	187	241	243

[a] See Table 1 for codes.
[b] Unique allele sizes in the table.

Table 3. Allele sizes (S) in bp and frequencies (%) of occurrences, for the six loci studied.

	VVS2		VVMD5		VVMD7		ZAG47		ZAG62		ZAG79	
	S	%	S	%	S	%	S	%	S	%	S	%
	130	21.4	218	8.9	231	0.9	151	2.7	185	16.1	241	5.4
	132	5.4	222	**27.7**	237	**46.4**	155	8.9	187	**33.9**	243	15.2
	134	4.5	224	3.6	241	11.6	157	**30.4**	191	1.8	245	**35.7**
	136	1.8	228	14.3	245	0.9	159	8.9	193	19.6	247	1.8
	140	22.3	230	6.2	247	9.8	161	12.5	195	13.4	249	**31.3**
	142	8.0	232	16.1	249	1.8	165	**31.3**	199	5.4	253	1.8
	148	1.8	234	16.1	251	4.5	171	5.4	203	9.8	255	3.6
	150	**28.6**	236	7.1	255	11.6					257	4.5
	154	1.8			261	12.5					259	0.9
	156	4.5										
Total:	**10**		**8**		**9**		**7**		**7**		**9**	
Hom.[a]:	**14.3%**		**12.5%**		**23.2%**		**16.1%**		**12.5%**		**25.0%**	

[a] Percentage of homozygosity in each locus.

obtained by Martín et al. (2003). Nineteen varieties have all the alleles heterozygous, other 34 have 1 or 2 homozygous alleles, while only three of them, Caíño Tinto, Caíño Longo and Tinto do Bragao have three homozygous loci.

The frequencies for each allele were calculated (Table 3). In all the studied loci there is at least one allele that is present in more than 25% of the cases. In comparison to a previous work with a broaden number of Spanish varieties (Martín et al. 2003), the highest frequencies in all the loci, except VVS2, occurred for the same alleles in all cases: 222 for VVMD5, 237 for VVMD7, 157 and 165 for ssrVrZAG47, 187 for ssrVrZAG62, and 245 and 249 for ssrVrZAG79, although the frequencies were generally lower than the ones obtained in that study. With respect to locus VVS2, the allele with the highest frequency (24.4%) was 130 in Martín et al. (2003), whereas it reached 21.4% in our case; in contrast, the allele 150 had a frequency of 14.8% instead of the 28.6% in Table 3. The overall comparison, however, leads us to the conclusion that the most frequent alleles are essentially the same as the ones in the previous study.

Based on the results of the STMS analysis, a double state (0,1) matrix was prepared in order to carry out the multivariate analysis and a two dimension grouping of the studied varieties was obtained. Figure 1 shows the resulting dendrogram of the 56 grapevine varieties.

This dendrogram shows the existence of five groups defined at the 0.33 similarity level. Group A includes 41 varieties and group B another 9 varieties. The formation of these two groups may be related to the origin of the varieties. There is no clear separation of both groups in relation to regions of origin.

Three varieties, Albillo Mayor, Tempranillo and Ferrón are somewhat distant from the two previous groups, while Moscatel de Grano Menudo, Tinto Gallego and Verdejo Colorado are markedly distant from the rest, probably because of a different origin.

At a similarity level of 0.83 (see Figure 1), three pairs of varieties are grouped: Albarín Negro and Carrasquín, both from Asturias and with black berries; Tinta Barroca and Touriga Francesa are both from the Douro region and have black berries; and Pedrol and Rabo de Ovelha Tinta both have black berries; in these cases, coincidence in 10 out of the 12 alleles occurs, always including at least one common allele from each locus. Consequently, these pairs of varieties are very likely highly related. Further morphological and molecular studies are needed in order to confirm and detect the genetic relationship between them.

At a level of 0.75, another 5 pairs of varieties show a similarity corresponding to coincidence in 9 alleles, and always including at least one common allele from each locus: Albarín Blanco and Puesto Mayor; Espadeiro and Merenzao; Cercial and Tinta Jeromo; Mencía and Tinta Carvalho; and Gajo Arroba and Juan García. Again, a marked relationship between each pair of varieties

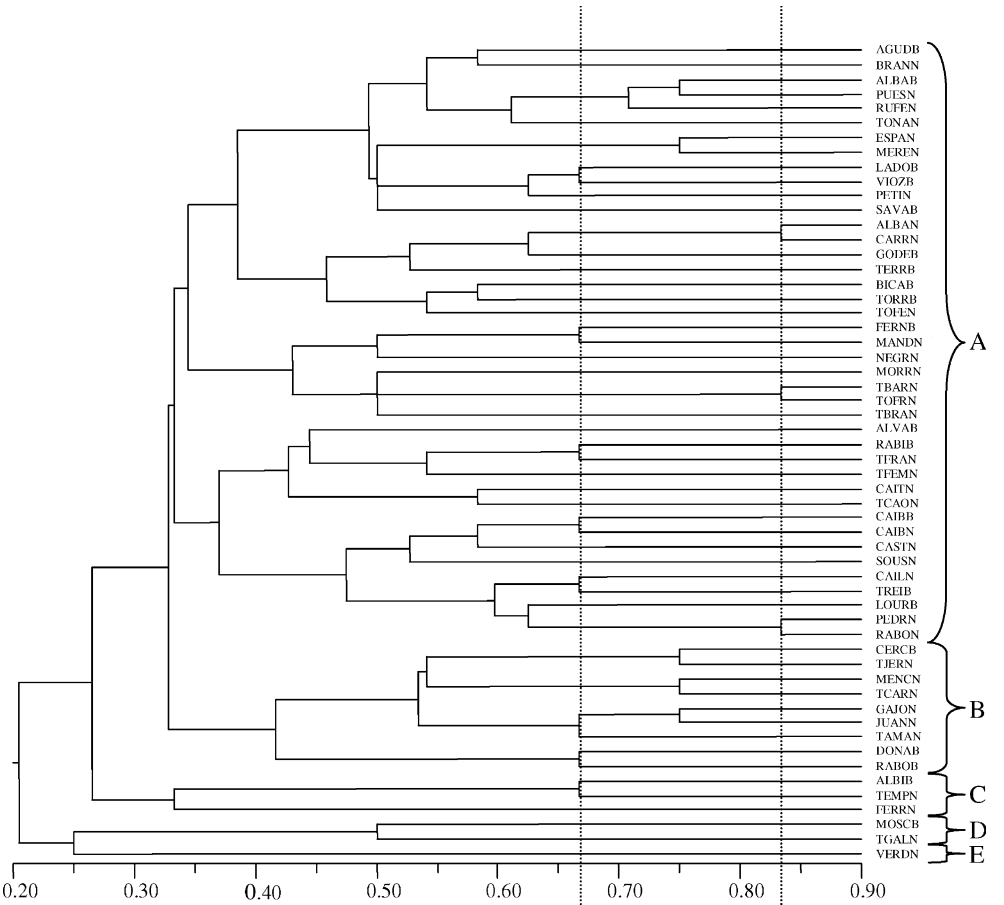

Figure 1. Dendrogram of the studied varieties obtained using Dice's similarity coefficient and UPGMA method. A–E, formed groups; see text for comments. Codes in Table 1.

is supposed, although some of these pairs include varieties with different berry colour.

At a level of 0.67, seven pairs of varieties show a similarity corresponds to coincidence in 8 alleles. Only 4 of these pairs include also at least one allele in common for each locus: Lado and Viosinho; Caíño Blanco and Caíño Bravo; Caíño Longo and Treixadura; and Albillo Mayor and Tempranillo. Relationship between these pairs of varieties is therefore supposed.

As a consequence of this study it can be concluded that the 272 studied accessions correspond to 56 different varieties. A marked variability from the morphological point of view has been observed among them. More complete ampelographic studies are currently being carried out. Conservation of this material is recommended in order to maintain a maximum variability for further breeding or commercial purposes.

According to the results it can be concluded that marked relationships exist among most of the studied varieties; only three to six varieties seem to be genetically distant from the rest, and this fact could be based on their external origin. A broader molecular study would give complementary information in order to detect the parentage relationships that may be present in the surveyed material. Inclusion of the accessions that are not jet in either of the collections, El Encín or Arcos de Valdevez (Table 1), is currently under way.

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