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Application of AFLPs to the characterization of grapevine *Vitis vinifera* L. genetic resources. A case study with accessions from Rioja (Spain)

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Abstract AFLPs were used to characterize 67 different grapevine accessions from a collection of D.O.Ca. Rioja in Spain. A correct selection of primers and selective nucleotides allowed us to maximize the number of amplified fragments analyzed per reaction yielding an average of 100 per reaction, 49% of which were polymorphic. Based on the presence or absence of amplified fragments for each genotype resulting from a reaction with two primer combinations, we have established the genetic similarity between the different accessions in the collection. These results allowed us to resolve different genotypes maintained under the same name (homonyms) and to identify the same genotype under different names (synonyms) thus permitting the elimination of redundant germplasm. Furthermore, by providing information on more than 50 polymorphic loci per reaction, a few reactions were sufficient to identify distinct AFLP patterns characteristic of specific clones, with different agronomic and organoleptic features, belonging to the same cultivar. The possibility for clonal identification, shown here for grapevines, can have important implications in the protection and management of clonal selections.

Key words *Vitis vinifera* · AFLPs · Cultivar identification · Clone identification

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Introduction

Many morphological and molecular markers have been used for the characterization of grapevine *Vitis vinifera* (L.) germplasm. Among them, ampelographic characterization according to morphological features (Galet 1979) has been useful in the identification of well-known grape varieties and has facilitated the clarification of ambigous denominations or the establishment of phenological relationships. Unfortunately, morphological characterization is a time-consuming process, is based on characters which can be affected by the environment (Levadoux 1956), and does not generally help to distinguish very close genotypes, such as clonal selections derived from a variety, or to predict genetic identity with a high probability. Other methods, based on the use of genetic variability at the level of proteins or nucleic acids, have frequently been used for these purposes with more or less success depending on the genetic relationships among the materials analyzed and the number of markers employed. From these studies, it is possible to conclude that the high genetic variability present in vegetatively reproduced grapevine cultivars allows their distinction with almost any molecular marker used, isozymes (Wolfe 1976; Schwennesen et al. 1982; Loukas et al. 1983; Stavrakakis and Loukas 1983; Bachmann and Blaisch 1988; Caló et al. 1989; Chaparro et al. 1989; Altube et al. 1991; Bachmann 1994; Cabello and Ortíz 1995), RFLPs (Striem et al. 1990; Bourquin et al. 1993; Bowers et al. 1993; Gogorcena et al. 1993), SSRPs (Thomas and Scott 1993; Bowers and Meredith 1994), RAPDs (Collins and Symons 1993; Jean-Jaques et al. 1993; Tschammer and Zyprian 1994; Grando et al. 1995, 1996; Lodhi et al. 1997), or, more recently, ISTRs and AFLPs (Sensi et al. 1996) as well as the 5′ untranslated regions of specific genes (Geuna et al. 1997). However, in many cases these methods do not have sufficient resolution to identify with enough certainty a specific cultivar as belonging to a given variety or to

distinguish clones that originated by somatic mutation or clonal selection and which belong to the same cultivar (Bowers et al. 1993; Botta et al. 1995).

La Rioja is an important wine producing area in Spain. The "Denominación de Origen Calificada Rioja" (D.O.Ca.), an administrative organization that controls the origin of grapes, their genetic background and their production, currently recognizes seven grape varieties for the production of Rioja wines, four red ones (Garnacha Tinta, Graciano, Mazuelo, and Tempranillo) and three white ones (Garnacha Blanca, Malvasía and Viura). The diminishing number of grown varieties, and the limited number of clones within them, implies a problem of loss of genetic variability. This is specially patent nowadays, when many of the old vines are being substituted by a few clones of the seven approved varieties. Six years ago the University of La Rioja, in collaboration with Bodegas Viña Ijalba S.A., started a project to characterize and preserve old genotypes that could represent valuable genetic combinations (Martínez de Toda and Sancha 1997a). This effort has resulted in the collection of more than 60 genotypes which are currently being characterized.

In the present work, our goal was to study the utility of AFLPs (Vos et al. 1995) in the identification of grapevine varieties and clones and in the management of germplasm collections. With this purpose in mind, we analyzed the grapevine varieties grown in Rioja, some of their clones, and most of the accessions corresponding to local isolates of grapevines preserved in the above-mentioned collection. Our results demonstrate the potential use of these molecular markers in the identification of grapevine genotypes. Using only two primer combinations, we were able to analyze presence or absence polymorphisms for 108 loci. This was enough to distinguish not only cultivars, but different clones belonging to the same cultivar. Moreover, the results allowed us to identify with high certainty cultivars that belong to the same variety within the collection, which will save time and reduce the cost of their conservation. Given the possibility of increasing the number of loci analyzed by increasing the number of primer combinations, the potential strength of this technology in cultivar and clone identification and in the management of grapevine germplasm collections is discussed with respect to previously used genetic markers.

Materials and methods

Plant material and DNA extraction

The grapevine accessions used in this study, together with their codes, local names and place of origin, are listed in Table 1. Representative cultivars of the varieties approved by the D.O.Ca. Rioja were also included in addition to other commercial cultivars. Leaf tissue of 67 accessions was obtained from grapevines grown at Bodegas Viña Ijalba S.A., Logroño, La Rioja (Spain). Total genomic DNA was isolated from young frozen leaves using the procedure described by Dellaporta et al. (1983). The extraction buffer was supplemented with 1% polyvinylpirrolidone to eliminate polyphenols (Lodhi et al. 1994).

AFLP protocol for grapevine

AFLP analysis was performed according to Vos et al. (1995) with the modifications described below. The DNA was digested using two restriction enzymes, *Mse*I (New England Biolabs) and *Eco*RI (Pharmacia). Digestion was carried out in a final volume of 35 µl in 10 mM Tris-HAc, 10 mM MgAc, 50 mM DTT, pH 7.5, 10 U of *Eco*RI, 8 U of *Mse*I and 500 ng of genomic DNA during 3 h at 37°C. Two different adapters, designed to avoid the reconstruction of these restriction sites, one for the *Eco*RI sticky ends and one for the *Mse*I sticky ends, were ligated to the DNA by adding to the digestion 5 µl of a mix containing 5 pmol of *Eco*RI adaptor, 50 pmol of *Mse*I adaptor, 8 mM ATP, 10 mM Tris-HAc, 10 mM MgAc, 50 mM DTT, pH 7.5 and 1.4 U of T4 DNA ligase (Boehringer). The ligation was incubated for 3 h at 37°C and overnight at 4°C. The *Eco*RI adaptor consisted of the combination of two primers: 5′-CTCGTAGACTGCGTACC and CTGACGCATGGTTAA-5′. The *Mse*I adaptor consisted of a combination of the primers: 5′-GACGATGAGTCCTGAG and TACTCAGGACTCAT-5′.

Digested-ligated DNA fragments were diluted 5-fold to be used as templates for the first amplification reaction, the pre-amplification step, prior to the selective radioactive PCR. The pre-amplification consisted of a PCR reaction using primers which are complementary to the adapters *Eco*RI and *Mse*I with an additional selective 3′ nucleotide. In this way, only 1/16 of the possible DNA restriction fragments are amplified. The PCR reactions were performed in a 20-µl vol of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each dNTP, 30 ng of each primer (GENSET) *Eco*RI + A and *Mse*I + C, 0.4 U of *Taq* DNA polymerase (Boehringer) and 3 µl of diluted fragments. The PCR amplifications were carried out in a Perkin Elmer 9600 using 28 cycles, each cycle consisting of 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C.

The pre-amplification products were diluted to be used as starting material for the selective radioactive amplification; thus, 10 µl of pre-amplified material were diluted by adding 180 µl of H_2O. For the selective radioactive amplification, only *Eco*RI primers were labelled; two *Eco*RI primers and one *Mse*I primer, containing the same sequences as those used in the pre-amplification but with three selective nucleotides at the 3′ end, were employed in each analysis selecting 1/128 of the pre-amplified fragments. The PCR reaction was performed in a 20-µl vol of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.08 mM of each dNTP, 4 ng of [^{33}P]-*Eco*RI primers, 24 ng of *Mse*I primer, 0.4 U of *Taq* DNA polymerase (Boehringer), and 5 µl of diluted pre-amplified DNA. The selective amplification was carried out using the following cycling parameters: 1 cycle of 30 s at 94°C, 30 s at 65°C, 1 min at 72°C followed by 12 cycles in which the annealing temperature decreases 0.7°C per cycle, followed by 23 cycles of 1 min at 94°C, 30 s at 56°C, and 1 min at 72°C. The start at a very high annealing temperature allows for optimal primer selectivity. By gradually decreasing the annealing temperature, the efficiency of primer binding increases (Don et al. 1991).

Two primer combinations were used in this analysis: 2 *Eco*RI (+ ACC, + ACT)/*Mse*I + CAT and 2 *Eco*RI (+ ACC, + ACT)/*Mse*I + CTG. At the end of the selective radioactive PCR, the samples were denatured by adding an equal volume of formamide-buffer (98% formamide, 10 mM EDTA, pH 8.0, 0.05% bromophenol blue and 0.05% xylene cyanol) and heated for 3 min at 94°C. Two to three microliters of each sample were loaded on 4.5% acrylamide/bisacrylamide 19 : 1, 7.5 M urea and 1 × TBE gels.

Table 1 Grapevine accessions analyzed. The question marks indicate that the name of the accession is unknown

Code	Local name	Origin	Code	Local name	Origin
Gar	Garnacha tinta	Bodegas Viña Ijalba	CI-78	Turruntés	CIDA – Mendavia
Garbl	Garnacha blanca	Bodegas Viña Ijalba	G-68	?	Grávalos
Grac	Graciano	Bodegas Viña Ijalba	I-57	Graciano	CIDA – Mendavia
Malv	Malvasía	Bodegas Viña Ijalba	I-58	Tinto Aragonés	CIDA – Mendavia
Maz	Mazuelo	Bodegas Viña Ijalba	I-59	Xarello	CIDA – Mendavia
Temp	Tempranillo	Bodegas Viña Ijalba	N-21	Cagazal	Nájera
Viur	Viura	Bodegas Viña Ijalba	N-22	Grano alargado	Nájera
A-15	Tempranillo temprano	Aldeanueva de Ebro	N-23	Tempranillo del barón	Nájera
A-16	Garnacha tintorera	Aldeanueva de Ebro	N-24	Blanca falsa	Nájera
A-17	Garnacha roya	Aldeanueva de Ebro	R-01	Morato	Alcanadre
A-18	Garnacha blanca	Aldeanueva de Ebro	R-02	Miguel de Arco	Alcanadre
A-20	?	Aldeanueva de Ebro	R-03	Tintorero	Alcanadre
A-34	Teta de vaca	Aldeanueva de Ebro	R-04	Moscatel de grano menudo	Alcanadre
AB-100	Turruntés	Abalos	R-05	?	Alcanadre
AR-36	?	Arnedo	R-06	?	Alcanadre
AR-37	?	Arnedo	R-08	Garnacha tardía	Galilea
AR-40	?	Arnedo	R-09	Malvasía	Galilea
AR-41	?	Arnedo	R-10	Graciano de Alfaro	Galilea
AR-43	Cojón de gato	Arnedo	R-11	Monastel	Alcanadre
AR-44	?	Arnedo	R-13	Cojón de gato	El Redal
B-46	?	Badarán	R-14	Sabor a menta	El Redal
B-48	?	Badarán	R-32	?	El Redal
B-49	Colgadera	Badarán	R-33	?	El Redal
B-50	Monastel	Badarán	RA-74	?	Granja Ramelluri
B-51	Monastel	Badarán	S-27	Silvestre hembra	Roncal
B-52	?	Badarán	SO-60	Ribadavia	Sotes
B-53	Graciano	Badarán	SO-61	Blancaza	Sotes
B-54	Blanca roja	Badarán	SO-62	Blanca alargada	Sotes
B-55	Moscatel de la tierra	Badarán	SO-63	Navarra	Sotes
B-56	?	Badarán	SO-64	Teta de vaca	Sotes
BE-69	Bobal	Baños de Ebro	SO-65	Tintorera	Sotes
BE-70	Graciano	Baños de Ebro	SV-28	Garchacha que no se corre	San Vicente de la Sonsierra
CI-75	Maturana blanca	CIDA – Mendavia	Porta	110-R	Commercial
CI-76	Maturana tinta	CIDA – Mendavia			

Data analysis

Only AFLP bands showing a clear polymorphism were scored as present (1) or absent (0). The genetic similarity (GS) between pairs was estimated according to Dice [Sneath and Sokal 1973; $GS(ij) = 2a/(2a + b + c)$] and Jaccard coefficients [Jaccard 1908; $GS(ij) = a/(a + b + c)$], where GS(ij) is the measure of genetic similarity between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. A dendrogram was constructed using the unweighted pair group method average (UPGMA) clustering of the NTSYS-PC software package, version 1.8 (Rohlf 1993).

Results

Previous experience in the selection of primer combinations for the analyses of woody plants (Cervera, unpublished results) was used to select the primers used in this study. Different numbers of selective nucleotides were tested, with the aim of obtaining an optimized number of scorable bands for every primer combination. As shown in Table 2, the combination of two *Eco*RI and one *Mse*I primers with three selective nucleotides each, gave the best results in terms of polymorphic scorable bands (classes 1 and 2) per gel. The results obtained for the two different primer combinations used in this study are shown in Table 3. A total of 116 and 104 bands were identified by the two combinations of primers employed. Of those, 64 and 44 respectively showed a clear polymorphism, representing 49.1% of the total bands, and were scored for their presence or absence in 67 grapevine accessions. Weak bands or bands showing more than two different intensities, which indicate the presence of more than one marker running at the same position (class-3 bands; 25 and 34 of the total bands respectively), were not considered in this study. The AFLP patterns shown by each cultivar are illustrated in Fig. 1, and were repeatedly found in different experiments using different vines belonging to the same accession.

The genetic similarity among the different accessions, based on the presence or absence of the amplified fragments, was calculated by Dice (Sneath and Sokal 1973) and Jaccard coefficients (Jaccard 1908). Using the data of genetic similarity, grapevine accessions were grouped in clusters as shown in Fig. 2. Either Jaccard

Table 2 Effect of nucleotide selection on the total number of amplified detectable and scorable fragments

Primer combination	Total bands	Total polymorphic bands (classes 1[a], 2[b] and 3[c])	Polymorphic bands (classes 1[a] and 2[b])	% Polymorphic bands (classes 1[a], 2[b] and 3[c])	% Polymorphic bands (classes 1[a] and 2[b])
E + AC/M + CTG	140	76	35	54.3	25
2E(+ ACC, + ACT)/ M + CTG	104	78	44	75.0	42.3
E + ACC/M + CTG	90	57	35	63.3	38.9

[a] Bands showing high intensity, easily scoreable
[b] Bands showing medium intensity, easily scoreable
[c] Weak bands or bands showing more than two different intensities indicating the presence of more than one marker running at the same position

Table 3 Total number of amplified fragments and polymorphic fragments detected with the primer combinations used in this study

Primer combination	Total bands	Total polymorphic bands (classes 1[a], 2[b] and 3[c])	Polymorphic bands (classes 1[a] and 2[b])	% Polymorphic bands (classes 1[a], 2[b] and 3[c])	% Polymorphic bands (classes 1[a] and 2[b])
2E(+ ACC, + ACT)/ M + CAT	116	89	64	76.7	55.2
2E(+ ACC, + ACT)/ M + CTG	104	78	44	75.0	42.3
Total	220	167	108	75.9	49.1

[a] Bands showing high intensity, easily scoreable
[b] Bands showing medium intensity, easily scoreable
[c] Weak bands or bands showing more than two different intensities indicating the presence of more than one marker running at the same position

or Dice coefficients of genetic similarity resulted in the same cluster order in the dendrogram and only the results obtained with Dice coefficient are presented in Fig. 2. Accession R-110, corresponding to a hybrid (*Vitis berlandieri* x *V. rupestris*) commonly employed as a rootstock, was used as a representative outgroup in the cluster analysis. Additionally, accession R14, that was also placed outside of the rest, corresponds to an accession, locally known as "sabor a menta" (mint flavor), that ampelographically corresponds to a putative hybrid (*V. labrusca* x *V. vinifera*). Apart from these two hybrids, the rest of the accessions showed different levels of similarity ranging between 0.70 and 1.00. A closer analysis of the dendrogram indicates that accessions typically considered as different varieties show similarities between 0.70 and 0.90, while accessions showing similarities higher than 90% can be considered as cultivars belonging to the same variety. For example, when the seven varieties approved by the D.O.Ca. Rioja (Garnacha Tinta, Garnacha Blanca, Tempranillo, Graciano, Mazuelo, Viura, Malvasía) were considered, they showed similarities always lower than 0.85, with the exception of Garnacha Blanca and Garnacha Tinta which, as expected from their putative clonal origin, showed a similarity higher than 0.90.

Furthermore, several accessions of the collection appeared to be highly related to those six varieties, showing genetic similarities around 0.90. The two primer combinations employed allowed the distinction of several accessions as cultivars of Garnacha (Accessions Gar, SV-28, A-17, Garbl), Graciano (Accessions Grac, B-53, AR-44, I-57, BE-70), Malvasía (Accessions Malv, B-54, R-09, SO-61) and Tempranillo (Accessions Temp, A-34, AR-40, R-32, I-58). Some of these accessions had already been given different local names like Blancaza (SO61) or Blanca Roja (B54) as compared to Malvasía (Malv, R-09), while in other cases the accessions corresponded to improved clonal selections like "Garnacha que no se corre" (SV-28). From a comparison of the dendrogram with the table of the presence-absence results we deduce that, in this experiment, one difference in the presence or absence of a single amplified fragment represents a dissimilarity ranging from 1.2 to 2.7%.

The analysis also allowed us to distinguish between accessions with genetic similarities lower than 0.90, in the range of different varieties which were given the same names, such as Garbl and A-18, both named as Garnacha Blanca, or Grac and R-10, both named as Graciano. In these two cases the differences found with AFLP analysis are in agreement with the distinction made by ampelographic analyses (Martínez de Toda and Sancha 1997a,b). Alternatively, AFLPs allowed us to prove that some accessions with different names,

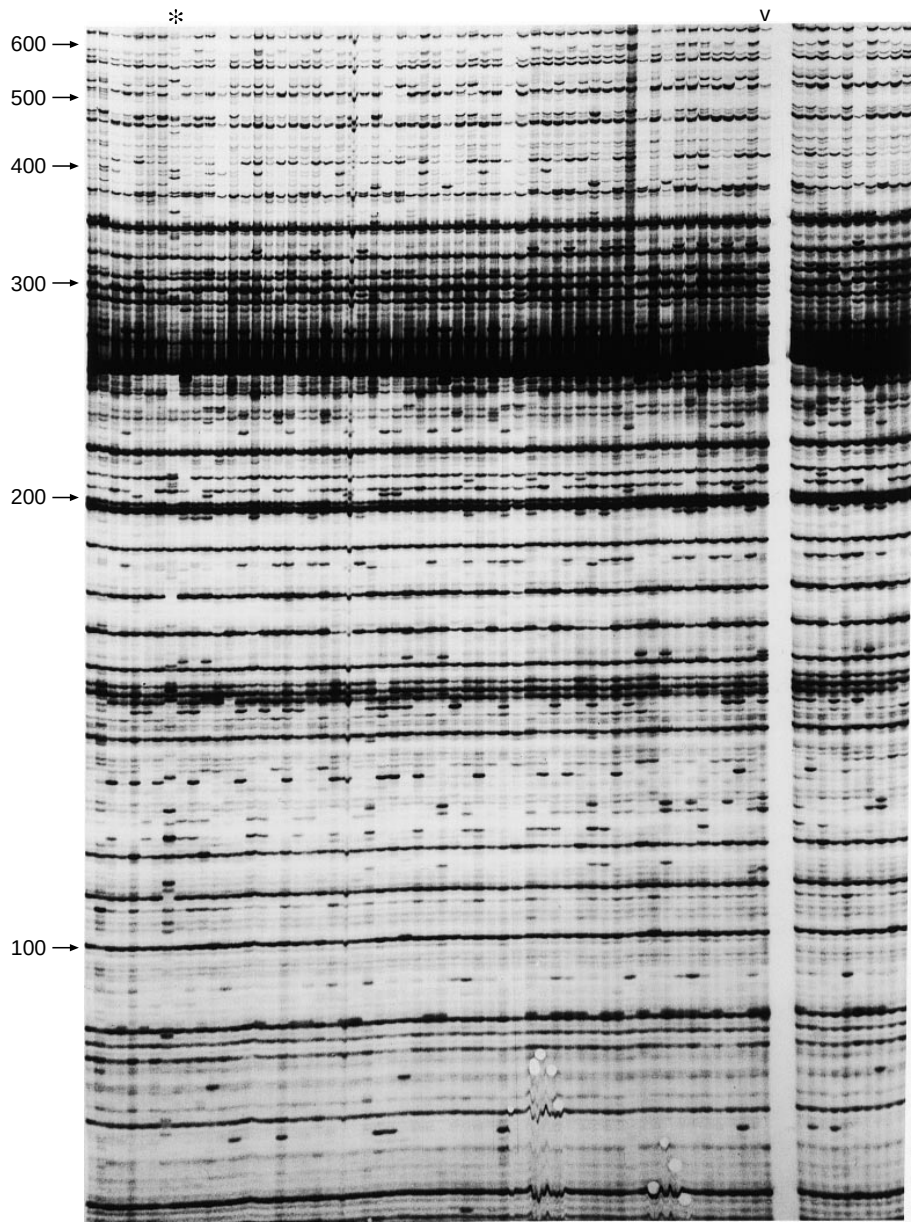

Fig 1 AFLP analysis of 67 grapevine accessions. The DNA fingerprints were generated using the primer combination 2 *Eco*RI (+ ACC, + ACT)/*Mse*I + CTG. The *arrows* indicate the size-marker positions. The * *lane* corresponds to the rootstock 110-R and the *V symbol* marks the lane corresponding to the putative hybrid *V. vinifera* x *V. labrusca*. The *empty lanes* were not loaded

which could have been considered as different cultivars, were closely related, showing a very similar or identical genotype (GS > 0.90). This was the case for accessions SO-60 and CI-75 (named as Ribadavia and Maturana Blanca), or accessions I-59 and N-24 (named as Xarello and Blanca falsa), which were identical for the 108 polymorphisms analyzed.

Finally, among the unidentified accessions within the collection, the results of AFLP analysis showed the genetic identity of accessions A-18 and R-33; AR-44 and I-57; AR-41, R-05 and R-13; or AR-40 and R-32. The probability that these identities were the result of chance is extremely low, given the number of loci scored. Thus, when identity is found with a known accession, it inmediately allows the identification of the unknown accession. The cluster analysis also revealed the existence of unknown accessions which could represent different varieties. This was the case for B-46, B-48, B-56 or RA-74, whose precise identification could be achieved through the genotyping of additional accessions of known name.

Discussion

Ampelographic markers together with different types of molecular markers have been used in the genetic identification of grapevine varieties and cultivars with different grades of success. In the present study we show that

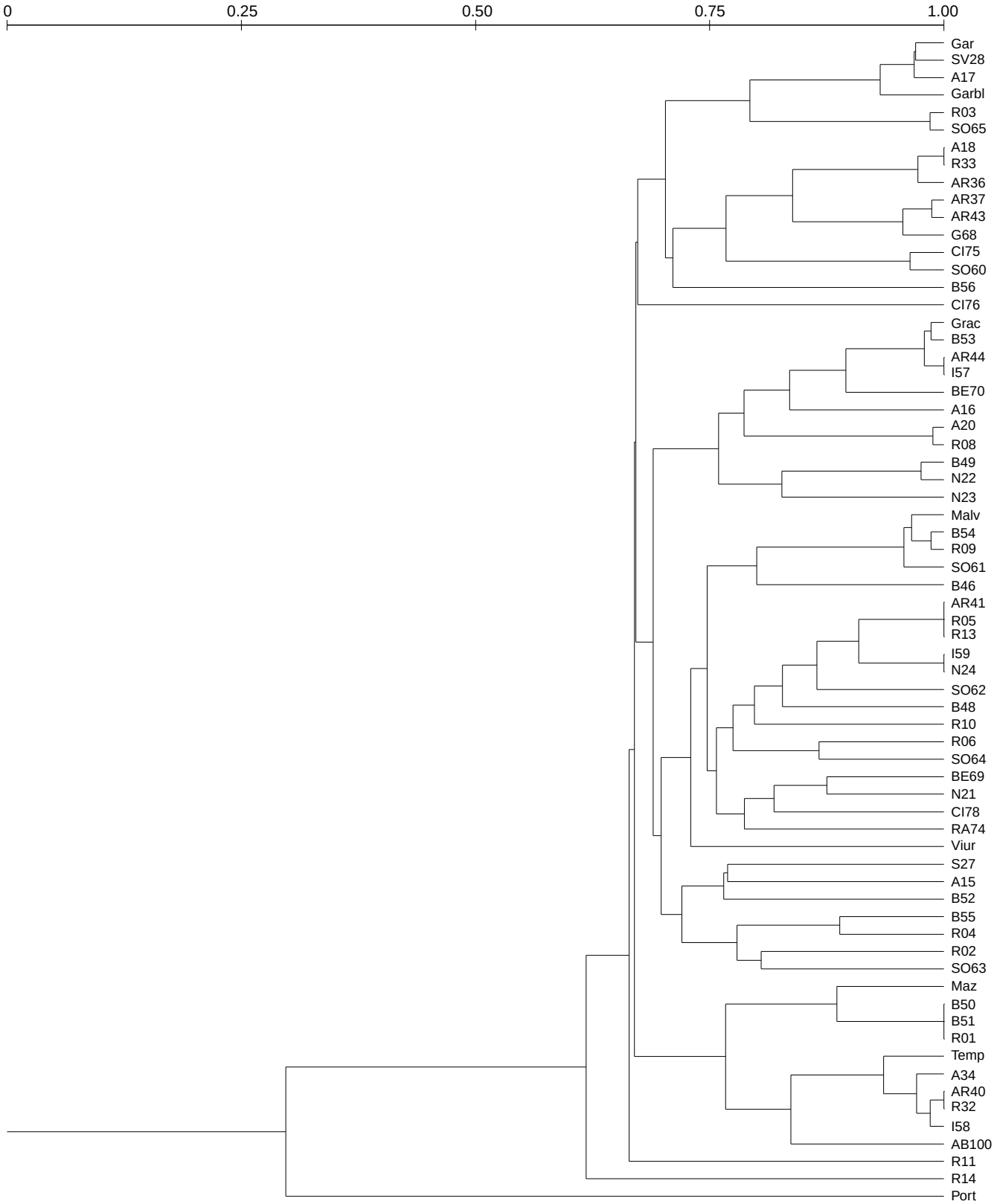

Fig 2 Dendrogram representing the genetic similarity among grapevine accessions. The dendrogram was constructed applying the UPGMA clustering method to the Dice estimates of genetic similarities based on AFLP analysis with two primer combinations

AFLPs, with specific conditions for selective amplification, can yield a large number of polymorphic bands per primer combination, being highly efficient in distinguishing grapevine cultivars. In fact, a single primer combination is enough to characterize the collection of accessions representing the approved varieties and most of the local cultivars and accessions of the wine producing area of D.O.Ca. Rioja. The use of a second primer combination helps to distinguish some of the selected clones within the cultivars. Additional primer combinations could also be used if distinctions between the remaining identical accessions were required.

These results are not surprising since other molecular markers also show high levels of polymorphism among grapevine varieties. Thus, RFLPs were also shown to be quite useful in distinguishing cultivars although they did not help to distinguish grapevine clones within the same cultivar (Bowers et al. 1993). RAPDs and SSLPs have also been used for cultivar identification although with similar drawbacks in the identification of clones within given cultivars (Gogorcena et al. 1993; Jean-Jaques et al. 1993; Thomas and Scott 1993; Bowers and Meredith 1994; Thomas et al. 1994). The added value of AFLP technology resides in two facts. First, a single primer combination is enough to ensure cultivar distinction, as it allows one to screen over 50 polymorphic loci. Second, the possibility of screening a higher number of anonymous loci than is possible with any other method makes AFLP more efficient to detect genetic differences among clones of the same cultivar. AFLP has recently been used to characterize genetic relationships among 16 cultivars of Sangiovese and Colorino using four pairs of primer combinations (Sensi et al. 1996). In that study AFLP proved to be less effective than "inverse sequence-tagged repeat analysis" (ISTR), most likely due to the excessive selection used in the selective amplification step of the AFLP technique. This excessive selection resulted in a reduced number of amplified fragments and polymorphic fragments per reaction. We show here how these results can be improved by varying the selection conditions.

Genetic similarity, measured on the basis of our AFLP results, generally agreed with the results of ampelographic analyses when the number of morphological characters considered was high (Martínez de Toda and Sancha 1997a,b). However, this agreement fades when ampelographic analyses are based on few characters, like berry colour that can vary between otherwise highly similar cultivars such as Garnacha Tinta and Garnacha Blanca. While ampelographic and AFLP determinations agree for very close or identical accessions (Martínez de Toda and Sancha 1997a,b), on less similar accessions the relationships established by AFLP markers can be much more reliable since they can be based on the analyses of a large number of unbiased genetic markers. These markers represent a random sample of genetic loci distributed along the genome, which decreases the variance of the similarity estimate (Cervera et al. 1996; Powell et al. 1996).

The use of AFLPs, allowing the screening of a large number of loci (several hundreds) with a few primer combinations, helps to rapidly identify unknown accessions and to establish genetic relatedness among them with high certainty. In our analysis, accessions like AR-41 and R-05 of unknown name were found to be identical to accession R-13 ("Cojón de Gato"), and accessions like AR-40 and R-32 were very close to I-58 (Tinto Aragonés). Although it is possible that the use of additional primer combinations may contribute to further distinction among accessions that appear to be genetically identical, our results (obtained after a comparison of 108 loci) strongly suggest a high degree of genetic similarity among them. The average probability for this identity to take place by chance alone is below 10^{-21} (Jeffreys 1987). Following a similar argument, it is highly probable that accessions standing apart from the others, like B-56, B-46, RA-74 or B-52, could represent different varieties. Therefore, this method provides a rapid, repetitive and efficient tool for the identification of germplasm, solving the problems generated by synonyms and homonyms. When complete identity is not found with respect to known accessions, the degree of genetic relatedness can help to elucidate if a specifc accession should be considered as a different variety or as a different cultivar within a given variety.

The demostration that AFLPs allow a distinction between different clones within a given cultivar is, perhaps, the most interesting result, not frequently reported until now, even with the use of SSLPs and RAPDs. This is most likely based on the high multiplex ratio of AFLPs (Powell et al. 1996) which allows the rapid screening of thousands of loci to identify sequence differences that could have accumulated since the separation of the clones. This distinction would therefore be easier for clones that have diverged for a longer time than for recently separated ones. Although the complexity of the AFLP analysis might preclude its general use in applied laboratories, clone-specific markers identified with this technology could later be transformed into SCARS (Paran and Michelmore 1993) to generate screening markers based on simpler PCR assays. These SCARS could then be used by other research groups and quality assessment centres to certify and protect the material delivered in the market. Furthermore, a close analysis of the AFLP patterns also allows for the identification of bands that are species-specific or bands that are conserved among different taxa of the genus *Vitis*. These markers point to conserved regions of the genome and could be used for taxonomic analysis above the species level. In this way the AFLP technology can be employed to identify specific and reproducible markers for their use at different taxonomic levels.

In this work we have demonstrated the utility of AFLPs for the examination of grapevine biodiversity and germplasm assessment. The possibility of screening a very large number of anonymous polymorphic loci, with very few reactions, opens the way to confirm identities with high certainty and to detect specific polymorphisms in order to differentiate clones, cultivars, varieties or species, something that could not be so far achieved with other markers. The use of two primer combinations has been effective enough to establish genetic relationships and to select a number of accessions which can be considered as identical so that redundant germplasm could be eliminated from the collection. Further characterization of additional grapevine cultivars and the establishment of a database will provide important information to assign unknown genotypes to previously known cultivars. Finally, the possibility of clonal identification through the development of clone-specific markers will require additional studies on the stability of these markers during clonal propagation. In conclusion the AFLP approach provides important practical advantages for DNA profiling and should play a major role in the efficient management of germplasm collections.

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