Molecular Marker Analysis of *Vitis vinifera* ‘Albariño’ and some Similar Grapevine Cultivars

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**Abstract.** ‘Albariño’ (*Vitis vinifera* L.) is an important grape cultivar in Spain, morphologically diverse but subject to much misnaming. The objectives of the present work were to correct some of the more common misnaminings concerning ‘Albariño’ and to evaluate the genetic variability within this cultivar by analyzing DNA polymorphisms using randomly amplified polymorphic DNA (RAPD) markers and microsatellite techniques. Several accessions of ‘Albariño’ (16 accessions from Misión Biológica de Galicia, one accession from El Encin, one accession from Rancho de la Merced), related cultivars (‘Alvarinho’, ‘Caiño blanco’, ‘Cainho branco’, ‘Loureiro’), and cultivars presumably identical to misnaminings (‘Savagnin blanc’ and ‘Gewürztraminer’) were analyzed using 20 RAPD markers and six microsatellite loci. Both techniques revealed polymorphism among ‘Albariño’, ‘Caiño blanco’, ‘Albariño’ from Rancho de la Merced and ‘Loureiro’. No polymorphism was detected among the 16 ‘Albariño’ accessions from Galicia, the ‘Albariño’ accession from El Encin and ‘Alvarinho’, nor among the ‘Albariño’ accession from Rancho de la Merced, ‘Savagnin blanc’ and ‘Gewürztraminer’, nor between ‘Caiño blanco’ and ‘Cainho branco’. These results enabled us to clarify the main misnaminings concerning these cultivars. The absence of polymorphism among the true ‘Albariño’ accessions did not allow the detection of any clonal variation. The suitability of both techniques for defining the cultivar level for grapevine is discussed.

‘Albariño’ is one of the most important grapevine (*Vitis vinifera*) cultivars grown in Galicia (northwest Spain) due to the high quality of the wine it produces. However, based on ampelographic data, confusion of the identity of ‘Albariño’ is common. In Spain, ‘Albariño’ and ‘Caiño blanco’ have sometimes been confused (Ferro, 1989). In the northern part of Portugal, a cultivar named ‘Alvarinho’ is grown. We believe that ‘Alvarinho’ and ‘Albariño’ are the same cultivar, as suggested by Truel (1983). The national Portuguese cultivar list (Oliveira e Silva, 1986), however, does not establish any relationship between these cultivars. Moreover, Cincannon da Costa (1900) considered ‘Alvarinho’ and ‘Cainho branco’ to be synonymous cultivars. Misnaming of other cultivars, such as ‘Savagnin blanc’, as ‘Albariño’ in grapevine repositories has also been reported (Truel, 1983). Several ampelographic studies (i.e., morphological and phenological analysis) have been documented but they do not always correspond (Descripciones Ampelográficas Nacionales, 1990; Pérez et al., 1993).

Although ‘Albariño’ is an important Spanish cultivar, no official references or any certified clones are available. To initiate clonal selection, ‘Albariño’ accessions from several areas of Galicia have been collected. The ampelographic analysis of these accesses has been performed (Loureiro, 1993; Martínez et al., 1994) and has revealed polymorphism at the morphological level, i.e., differences in leaf morphology, size, density, and bunch shape. Variation between clones of the same cultivar (Rives, 1981) is common for grapevine: differences at the phenotypical level have already been reported for many cultivars such as ‘Pinot noir’ (Bernard and Leguay, 1979) and ‘Cabernet Sauvignon’ (Leclaire, 1995). In the case of ‘Albariño’ accessions, however, the existence of clones was difficult to certify by ampelography. The number of differences encountered were significant, and it was therefore difficult to accept that all accessions were really ‘Albariño’, but not large enough to enable the assignment of any accession to other known varieties. The existence of accessions obtained through hybridization was still possible.

The development of a more objective way to discriminate different cultivars, clones of the same cultivar, or seedlings obtained by hybridization or selfing is therefore of great importance. DNA markers provide a complementary approach for the distinction of grape cultivars (Boursiquot and This, 1996; Thomas et al., 1993). Randomly amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990) have proven useful for analyzing genetic relationships and identifying several species such as sweetpotato (Connolly et al., 1994), mango (Schnell et al., 1995), and celery (Yang and Quiros, 1993). Microsatellite markers (Beckmann and Soller, 1990) have also been successfully applied to identify several species such as potato (Kawchuk et al., 1996), hops (Brady et al., 1996), and Sitka spruce (Vandeven and McNicol, 1996). Microsatellite markers have been developed for grapevine (Bowers et al., 1996; Thomas and Scott, 1993). These two techniques (RAPD markers and microsatellites) have been used successfully for identifying grape cultivars (Botta et al., 1995; Büscher et al., 1993; Cipriani et al., 1994; Moreno et al., 1995; Thomas et al., 1994; Vignani et al., 1996) but have so far failed to...
identify any clonal variation other than somaclonal polymorphism (Schneider et al., 1996).

Several accessions of 'Albariño' from Spain, 'Alvarinho', 'Caño blanco', and 'Caño branco' (cultivars often referred as mismomers), other cultivars grown in the same area ('Loureiro'), and cultivars presumably identical to some mismomers ("Savagnin blanco", 'Gewürztraminer') were analyzed using RAPD and microsatellite markers. The objectives of the present work were to analyze the polymorphism at the DNA level among these accessions to 1) elucidate some of the most common mismomers concerning 'Albariño' and 2) evaluate the variation at the DNA level within the 'Albariño' accessions to confirm their identity as 'Albariño'.

**Material and Methods**

**Plant Material.** Twenty-four accessions were analyzed in this study (Table 1). Cuttings 40 cm long were collected from the different germplasm collections in the vineyard, after leaf fall, in January 1996. They were stored at 4 °C in plastic bags until further use. The 16 accessions from Misión Biológica de Galicia were collected in different locations in Galicia (Loureiro, 1993; Martínez et al., 1994); they will be further referred to as 'Albariño'. The 'Albariño' accession from El Encin collection will be further referred to as AlbariñoEE, whereas the 'Albariño' accession from Rancho de la Merced will be referred to as AlbariñoRM. For two accessions (Table 1), young leaves were collected at the Domaine de Vassal (France), frozen in liquid nitrogen, and stored at −20 °C until use.

**DNA Extraction.** DNA was extracted directly from 2 g of wood or 2 g of leaves according to the technique described by This et al. (1997). After extraction, DNA was resuspended in 0.4 mL of TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA) and quantified on 0.8% agarose gel stained with ethidium bromide by visual comparison with known quantities of lambda DNA (Boehringer Mannheim GmbH, Mannheim, Germany.)

**RAPD Analysis.** The method described by This et al. (1997) was used for the amplification reactions. A thermocycler (Trio-thermobloc; Biometra, Göttingen, Germany) was programmed for one step of 4 min at 94 °C, followed by 36 cycles of 1 min at 94 °C, 1 min at 38 °C, 1 min at 72 °C, and a final step of 6 min at 72 °C, using the fastest possible transition between each temperature. Another thermocycler (Biomed; Theres, Germany) was pro-

![Figure A](image1.png)

**Figure A.** RAPD profiles obtained using primer opP10 (A) and opP02 (B) with DNA of different accessions. Selected markers are indicated by white arrows. Size of the markers are in bp; 1-kb ladder = molecular size marker, control = control without DNA.
programmed using the same cycles except that the time of the denaturation, annealing, and extension phases were 1 min 15 s instead of 1 min.

Eight microliters of the amplified products was analyzed by electrophoresis in 1.6% agarose gel in 0.5x TBE (45 mm Trisborate, 1 mm EDTA) at 4 V per cm for 5 h along with a molecular size marker (1-Kb ladder; Life Technologies Inc., Gaithersburg, Md.). Bands were detected after staining with ethidium bromide and gels were photographed under UV light. Primers were purchased from Bioprobe (Montreuil sous Bois, France) using the same code as Operon (Alameda, Calif.) for the same sequence. Twenty oligonucleotides from kits A, B, C, D, E, P, and O (opA07, opA09, opB01, opC04, opC05, opC06, opC07, opC08, opD15, opD16, opD18, opE17, opE18, opE19, opP02, opP08, opP10, opP16, opP17, and opP02) were selected for analysis because they yielded a high number of well-separated and intense bands.

Microsatellite Analysis. Six microsatellite loci were analyzed: VVS1, VVS2, VVS29 (Thomas and Scott, 1993, Thomas et al., 1994), VVMD5, VVMD6, and VVMD7 (Bowers et al., 1996). The analysis was performed according to Bowers et al. (1996) with the following modifications: each reaction volume of 25 μL included 0.2 U of Taq DNA polymerase (Appligene-Oncor, Illkirch, France), 1x buffer (10 mm Tris-HCl pH 9.0, 50 mm KCl, 0.02% or 0.01% (w/v) gelatin, 1.5 mm MgCl₂, 0.1% Triton X-100) provided with the polymerase, 200 μm each of dATP, dCTP, dGTP, and dTTP (Boehringer Mannheim), 10 pmol of primer, and 25 ng of template DNA. During the manipulations, the tubes were kept on ice. The reaction was overlaid with one drop of mineral oil. Tubes containing all reaction components except for the DNA template were included as controls. A Biomed thermocycler was programmed for one step of 3 min at 94 °C followed by 26 cycles of 1 min 15 s at 94 °C, 1 min 15 s at 55 °C, 1 min 15 s at 72 °C, and a final step of 5 min at 72 °C, using the fastest possible transition between each temperature.

After amplification, an equal volume of loading buffer (95% formamide, 0.5% bromophenol blue, 0.5% xylol blue, 10 mm EDTA) was added to each sample. Four microliters of each sample was then loaded on a sequencing gel (6% acrylamide gel, 7.5 mm urea in 1x TBE). Electrophoresis was carried out at 35 mA for 2 h. The gel was then stained according to the protocol of the Promega (Madison, Wis.) silver staining kit.

RAPD Data Analysis. Only intense RAPD bands were scored 1 for presence and 0 for absence and reported in a binary matrix. The scoring did not consider any differences in intensity of the bands among profiles from different samples. The Jaccard (1908) and simple matching (Sokal and Michener, 1958) coefficients of similarity (CJ, CSM) and the corresponding distances (DJ = 1 – CJ; DSM = 1 – CSM) were calculated. The distance matrices were analyzed by the NEIGHBOR program of PHYLIP (Felsenstein, 1989) using the unweighted paired group method of arithmetic averages (UPGMA method, Sneath and Sokal, 1973). The trees were generated by the DRAWGRAM program of PHYLIP.

Results

RAPD Analysis. The analysis of the 24 accessions was performed using the 20 selected primers. An example of the patterns obtained with primers opP10 and opP02 is presented in Fig. 1. Amplifications were performed at least once with all samples but

Fig. 2. Polymorphic bands identified by RAPD with 17 of the selected primers on several accessions. A dark-grey box represents the presence of the marker, the white box represents the absence of the marker.
were repeated for some of them. Whenever a polymorphism between the 'Albariño' samples was detected, the amplification was repeated with the same DNA along with DNA from separate extraction. All of the selected primers produced profiles with well-separated bands, and 150 intense bands were observed.

No reproducible polymorphism was observed among the 'Albariño' clones (Figs. 1 and 2). In the subsequent analysis, we therefore considered only one genotype of 'Albariño'. No polymorphism was detected between the 'Albariño' clones and AlbariñoEE or 'Alvarinho'. Polymorphism between the remaining accessions was then analyzed. No polymorphism was detected between 'Caño blanco' from Galicia and 'Cainho branco' from Portugal, or between AlbariñoRM and 'Savagnin blanco' or 'Gewürztraminer' (Fig. 2). Among these three latter samples, however, differences in intensity were observed for one or two bands (Fig. 3) on the profiles obtained with several primers (opB01, opB17, opC04, opC06, opC08, opE18, opE19, opP02, and opP17). These differences were reproducible between the repetitions. Therefore, only polymorphic bands between 'Albariño', 'Caño blanco', AlbariñoRM, and 'Loureiro' were considered as markers. Sixty-three bands from 17 primers were identified as markers (Fig. 2). The number of markers per primer ranged from one to seven with a mean number of 3.7 markers per primer. Among the markers, 24 (38%) were specific to one cultivar (9 specific to 'Loureiro', 11 specific to AlbariñoRM, 4 specific to 'Albariño', but none to 'Caño blanco').

**RAPD DISTANCES.** The genetic distances among the nine accessions ('Albariño', AlbariñoEE, 'Alvarinho', 'Caño blanco', 'Cainho branco', AlbariñoRM, 'Savagnin blanco', 'Gewürztraminer', and 'Loureiro') were calculated from RAPD data with the 63 markers using Jaccard and simple matching distances (Table 2). Smaller distances were observed between 'Albariño' and 'Caño blanco' (DJ = 0.634, DSM = 0.413) and larger distances were observed between 'Loureiro' and AlbariñoRM (DJ = 0.815, DSM = 0.698). If we had taken into account all the intense bands (including monomorphic ones), distances would have been significantly lower (between 0.173 and 0.293 for DSM and between 0.215 and 0.272 for DJ). Trees were drawn from the cluster analysis performed using the UPGMA method from distances calculated with polymorphic bands. They were identical with both distance formulas. 'Albariño', AlbariñoEE, and 'Alvarinho' were on the same branch as well as 'Caño blanco' and 'Cainho branco' or AlbariñoRM, 'Savagnin blanco', and 'Gewürztraminer' (Fig. 4). The 'Albariño' branch and 'Caño' branch clustered together, while the AlbariñoRM branch clustered between this first cluster and 'Loureiro'.

**MICROSATELLITE ANALYSIS.** The microsatellite analysis was performed using the six loci on the 24 accessions. The number of alleles per locus ranged from two (VVS29) to five alleles (VVM7). Heterozygosity among the cultivars was high since 66% to 83% of the loci were heterozygous for each accession tested (Fig. 5). No polymorphism was observed between the accessions of 'Albariño' and AlbariñoEE nor with 'Alvarinho' (Fig. 6). No polymorphism was detected between 'Caño blanco' and 'Cainho branco' nor between AlbariñoRM, 'Gewürztraminer', and 'Savagnin blanco' (Fig. 5). On the contrary, polymorphism between 'Albariño', AlbariñoRM, 'Caño blanco', and 'Loureiro' was high since they had different profiles for the six loci. 'Loureiro' and 'Caño blanco' had identical alleles at three out of the six loci (Fig. 5).

**Discussion.**

**POLYMORPHISM REVEALED AMONG THE ANALYZED CULTIVARS.** Polymorphism detected between 'Albariño', 'Caño blanco', and 'Loureiro' was high since 42% of the RAPD bands were polymor-

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**Table 2. Genetic distances between the cultivars calculated from RAPD data.** On the upper corner, simple matching distances and on the lower corner, Jaccard distances (underlined).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Alb 1-16</th>
<th>Alb EE</th>
<th>Alv</th>
<th>Alb RM</th>
<th>Sav</th>
<th>Gew</th>
<th>Cain</th>
<th>Cain B</th>
<th>Lou</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alb 1-16</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0.508</td>
<td>0</td>
<td>0.508</td>
<td>0.508</td>
<td>0.413</td>
<td>0.413</td>
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<tr>
<td>Alb EE</td>
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<td>0</td>
<td>0.508</td>
<td>0</td>
<td>0.508</td>
<td>0.508</td>
<td>0.413</td>
<td>0.413</td>
</tr>
<tr>
<td>Alv</td>
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<td>0</td>
<td>0</td>
<td>0.508</td>
<td>0</td>
<td>0.508</td>
<td>0.508</td>
<td>0.413</td>
<td>0.413</td>
</tr>
<tr>
<td>Alb RM</td>
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<td>0.681</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.571</td>
<td>0.571</td>
<td>0.698</td>
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<tr>
<td>Sav</td>
<td>0.681</td>
<td>0.681</td>
<td>0</td>
<td>0.571</td>
<td>0</td>
<td>0.571</td>
<td>0.571</td>
<td>0.698</td>
<td></td>
</tr>
<tr>
<td>Gew</td>
<td>0.681</td>
<td>0.681</td>
<td>0</td>
<td>0.571</td>
<td>0</td>
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<td>0.571</td>
<td>0.698</td>
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<tr>
<td>Cain</td>
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<td>0.634</td>
<td>0</td>
<td>0.571</td>
<td>0</td>
<td>0.571</td>
<td>0.571</td>
<td>0.698</td>
<td></td>
</tr>
<tr>
<td>Cain B</td>
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<td>0.634</td>
<td>0</td>
<td>0.571</td>
<td>0</td>
<td>0.571</td>
<td>0.571</td>
<td>0.698</td>
<td></td>
</tr>
<tr>
<td>Lou</td>
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<td>0.731</td>
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<td>0</td>
<td>0.815</td>
<td>0.815</td>
<td>0.739</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a}\)Codes according to Table 1.

\(^{b}\)Alb1-16 correspond to the 16 accessions from Mision Biologica de Galicia.
phic with a high percentage of markers specific to only one cultivar and these cultivars were differentiated with the microsatellite loci. This polymorphism is however lower than the polymorphism previously detected by RAPD in an interspecific study of grape (Qu et al., 1996). Genetic distances calculated on polymorphic RAPD bands were therefore high (between 0.413 and 0.698 for DSM and between 0.634 and 0.815 for DJ) and much higher than Nei and Li's genetic distances among 33 grapevine cultivars calculated on 49 RFLP bands (Bowers and Meredith, 1996). However, many monomorphic bands were observed (83 bands), which might have been polymorphic if more cultivars had been analyzed. Genetic distances should therefore be considered as smaller (<0.293). Although the clustering obtained with RAPD data was based on distances calculated considering only the polymorphic bands, it would probably have been similar if monomorphic bands had been included, as demonstrated in peaches by Warburton and Bliss (1996). Clusters obtained by both distance formulas on RAPD data were in accordance with the ampelographic data from these cultivars. 'Albariño' and 'Caño blanco' both have small leaves with almost no sinus and a high density of prostrate hairs on the lower side of the leaves (Loureiro, 1993). Likewise, 'Gewürztraminer' and 'Savagnin blanc' also have small hairy leaves with small sinuses (Galet, 1990; Truel, 1983). In contrast, 'Loureiro' has larger leaves with well-developed sinuses (Pérez et al., 1993). Interestingly, based on the microsatellite analysis, 'Loureiro' seems closer to 'Caño blanco' than 'Albariño'. However, to clarify this difference between microsatellite and RAPD markers, the analysis should be extended by using more microsatellite loci.

**CORRECTING MISTAKEN BETWEEN CULTIVARS.** Both molecular techniques have been useful for discriminating the cultivars and elucidating some misnaming concerning 'Albariño'. AlbaríñoRM was different from the other 'Albariño' accessions tested. Considering that it could be 'Savagnin blanc' (Truel, 1983), DNA profiles were compared to those of 'Savagnin blanc' and 'Gewürztraminer', a colored and aromatic mutant of 'Savagnin blanc' (Bailhazard, 1983; Galet, 1990). No RAPD or microsatellite polymorphism was detected between AlbaríñoRM and 'Savagnin blanc' or 'Gewürztraminer'. Only a difference of intensity in some RAPD bands was detected between AlbaríñoRM profiles and the profiles of the two other cultivars, as already observed among 'Pinot noir', 'Pinot gris', and 'Pinot blanc' by Gogorcena et al. (1993). This difference was reproducible when the reactions were duplicated. DNA from AlbaríñoRM was extracted from the wood, whereas DNA from 'Savagnin blanc' and 'Gewürztraminer' was extracted from leaves, which could have led to these differences. In any case, this similarity of profiles is a strong argument supporting the hypothesis that they are the same cultivar considering the high number of primers tested. The hypothesis drawn by Truel (1983) is therefore confirmed. The misnaming of this accession might have originated during plant material transfers (from Galicia to Sancho de la Merced via El Encín). AlbaríñoEE is a true 'Albariño'.

Considering the molecular analysis and the ampelographical data, we can also conclude that 'Caño blanco' from Galicia is identical to 'Caño blanco' from Portugal. Since 'Caño blanco' is mostly cultivated in the northern part of Portugal and 'Caño blanco' is only cultivated in the El Rosal region of Spain (directly across the border from northern Portugal), 'Caño blanco' is more likely of Portuguese origin. Likewise, the results obtained in this study clearly indicate that 'Albariño' in Spain is identical to 'Albarino' in Portugal. 'Albariño' (or 'Alvarinho') and 'Caño blanco' (or 'Caño blanco'), which are morphologically very close, were often mistaken for, or even considered to be, the same cultivar (Cincinnati da Costa, 1900; Ferro, 1989). However,
considering the molecular analysis, we should consider them as distinct cultivars. This has economically important implications since these cultivars produce different kinds of wine.

**Polymorphism within cultivars.** No polymorphism at the DNA level was detected by either technique between the 16 'Albariño' accessions collected in Galicia. Nonreproducible polymorphic bands have been observed between these samples, but they were either amplification artifacts (not reproduced between amplifications) or extraction artifacts or contaminants (not reproduced between amplification using DNA from different extractions). When dealing with clonal variation, we therefore recommend duplicating the DNA extractions whenever differences are encountered.

Identity at the DNA level between the 'Albariño' accessions observed in this study allowed us to conclude that they all belong to the same cultivar, considering the high number of tested primers and that they do not correspond to closely related cultivars. It is also highly unlikely that they correspond to seedlings obtained by hybridization or selfing, since the level of heterozygosity of the grapevine genome is very high (Bowers et al., 1996; Thomas et al., 1993). More accessions from this region, including any official reference, should be analyzed before we can conclude that they represent the true 'Albariño' type.

As no polymorphism was detected, it was not possible to differentiate any clones among the 'Albariño' accessions. However, the clear differences of leaf morphology (Loureiro, 1993; Martínez et al., 1994), bunch shape, and density detected could indicate that the accessions corresponded to different clones. The absence of polymorphism thus suggests that more analysis is needed or that both RAPD and microsatellite makers are inadequate to detect clonal polymorphism as already indicated (Botta et al., 1995; Cipriani et al., 1994; Gogorcena et al., 1993; Vignani et al., 1996). The differences observed between the accessions could however be of other origin, as indicated by Boidron (1995), and more analysis will be required before we conclude to the existence or nonexistence of 'Albariño' clones.

Nevertheless, both RAPD and microsatellite makers are very useful as a more objective way to determine the varietal level between different accessions: whenever polymorphism between two samples can be detected with RAPD primers and microsatellite loci, one can then conclude that they are two different cultivars. However, more analysis of clonal polymorphism and analysis of many closely related cultivars is necessary to determine the minimum number of RAPD primers or microsatellite loci that are needed before we can ascertain the identity and the level of significance of the analysis.

Although 'Albariño' is one of the most important cultivars in Galicia, no certified clones are available, since clonal selection has never been attempted and virus infections are common (Segura et al., 1991). We have demonstrated that the different 'Albariño' accessions from Galicia and El Encín belong to the same cultivar and, since there are morphological and agronomical differences, it is now possible to initiate clonal selection. This selection would enhance the sanitary and qualitative level of 'Albariño' as it has already been demonstrated in France for several cultivars (Boix et al., 1995) and would surely benefit Galician viticulture.

**Literature Cited**


