Morphological and Molecular Characterization of Grapevine Accessions Known as Albillo

M.T. Cervera,[1] I. Rodriguez,[2] J.A. Cabezas,[1] J. Chavez,[2] J.M. Martínez-Zapater,[1]* and F. Cabello[2]

Twenty-eight accessions identified under the name of Albillo or cited as a synonym of this ancient Spanish grapevine variety were characterized using 43 morphological descriptors, four different isozyme systems (CO, ACPH, GOT, and SOD) and two Amplified Fragment Length Polymorphism (AFLP) primer combinations. Genetic similarity data resulting from these analyses allowed three different groups of Albillo to be distinguished that corresponded with geographical and historical origin of representative varieties: Turruntés, Malvasía de El Bierzo, and the classical Albillo de Madrid, related to the oldest Albillo ampelographical descriptions. In addition, homonyms as well as varieties incorrectly cited as Albillo synonyms were identified. To ensure accurate variety identification, association among historical descriptions based on morphological descriptors and molecular fingerprints was required. Isozymes proved to be useful markers for varietal identification. However, only AFLP allowed distinction among closely related varieties as well as the analysis of intravarietal variation.

Key words: Ampelography, isozymes, AFLP, variety characterization

Grapevine cultivation in the Iberian Peninsula dates back to Roman times. Imported germplasm resulting from expanding trade routes and demographic migration further increased genetic diversity [17]. The practice of vegetative propagation has preserved original varieties until today. However, original names may have been confused and/or forgotten and it is not uncommon to find groups of very different varieties that are given the same name (homonymies) as well as different names being given to the same variety in different regions (synonymies) [10]. Ampelography and ampelometry techniques as well as isozyme markers approved by the Office International de la Vigne et du Vin (OIV) [26] are commonly used to identify and clarify cases of synonymies and homonymies. More recently, new techniques based on the use of DNA polymorphism analysis have also been used to further this goal (RFLPs [4,6], RAPDs [11,13,23,34], microsatellites [5,31,35,36], ISTRs [24,32], and AFLPs [8,9,16,32]).

Albillo is a classical variety, only grown in Spain [27]. Although the grapevine and its wine were described in 1513 by Alonso de Herrera in *Agricultura General* [1], the origins of Albillo are unknown. In fact, the name *Albillo* has been given to many different accessions with white, small, and very aromatic fruits that mature early in the season. Later descriptions of Albillo recognized the existence of different Albillos named according to their origin [10,15,19]. The early season of Albillo and the quality of its grapes are appreciated in both table grape and wine use. Although the generation of new table grape varieties is reducing Albillo's use as fresh fruit, its flavor and the quality of its wines are very much appreciated in some growing areas such as the Denomination of Origin (DO) Vinos de Madrid, Ribera del Duero, and Bierzo. Increased use of Abillo for either wine or table grapes requires clear characterization of grapevines grown under this name in Spain. With this purpose we have used ampelographic and molecular markers, including isozymes and AFLPs, to identify the different genotypes sharing this common name. Results provide clear conclusions about the genetic variability within Albillo and its similarities to other varieties in Spain. Moreover, they demonstrate the validity of classical methods, morphology or isozyme analysis, to characterize well-established varieties and affirm the usefulness of AFLP in identifying similarities of closely related accessions while providing clues on putative genetic relationships frequently overlooked.

Material and Methods

Plant material. Twenty-eight accessions of *Vitis vinifera* were used. Their codes, local names, date of receipt, and place of origin are listed in Table 1. The study also included one sample, Richter 110, corresponding to a hybrid (*Vitis berlandieri* x *V. rupestris*), commonly used as rootstock and used as an outgroup in the AFLP analysis. Representative individuals of these varieties are maintained in the collection of El Encín in Alcalá de Henares (Spain), which belongs to the Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA) de la Comunidad de Madrid. Grapevines at El Encín are grown on the second ter-

[1]Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, CSIC, Campus de la Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain and Departamento de Mejora Genética y Biotecnología, SGIT, INIA, Ctra. de la Coruña Km 7, 28049 Madrid, Spain. [2]Sección de Viticultura y Enología, Finca El Encín, IMIA, Apdo 127, 28800 Alcalá de Henares, Madrid, Spain.

*Corresponding author: [Tel: 34-91-585-4687; Fax: 34-91-585-4506; email: zapater@cnb.uam.es]

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Table 1 Grapevine accessions analyzed.

Sample	Code	Local name	Date of receipt	Synonyms	Author of synonym description	Origin
1	D-58	Nieves Temprano	01/01/14	Albillo	Borrego, J. (1990)	Zaragoza (Z)
2	F-31	Albillo	02/15/71	Albillo		Oviedo (O)
3	G-06	Albillo Blanco	01/03/72	Albillo	Borrego, J. (1990)	Bilbao (BI)
4	H-49	Albillo	01/01/42	Albillo		Albacete (AB)
5	I-16	Albillo	01/01/51	Albillo		Avila (AV)
6	I-47	Albilla	01/27/80	Albillo	Clemente, S. (1807)	Guadalajara (GU)
7	J-15	Albillo	01/01/42	Albillo		Madrid (M)
8	J-21	Temprano de Campo Real	01/01/42	Albillo	Cabello, F. (1992)	Madrid (M)
9	J-43	Temprano de Mora	01/01/42	Albillo	Cabello, F. (1992)	Toledo (TO)
10	K-01	Albillo	03/11/80	Albillo		Burgos (BU)
11	K-02	Blanco del Paĺs	10/03/80	Albillo	Borrego, J. (1990)	Burgos (BU)
12	K-27	Albillo	12/18/74	Albillo		León (LE)
13	K-49	Albillo	11/16/76	Albillo		Salamanca (SA)
14	K-56	Castellana Blanca	02/26/71	Albillo	Baccius	Segovia (SG)
15	K-58	Albillo	04/01/83	Albillo		Segovia (SG)
16	L-23	Albillo	02/06/71	Albillo		Valladolid (VA)-1
17	L-38	Albillo	01/29/73	Albillo		Valladolid (VA)-2
18	L-58	Albillo	01/14/80	Albillo		Zamora (ZA)
19	M-03	Albillo de Toro	01/01/42	Albillo	Borrego, J. (1990)	Zamora (ZA)
20	M-35	Albillo Real	01/01/42	Alarije	Cabello, F. (1992)	Cáceres (CC)
21	O-14	Blanco Castellano	01/01/14	Albillo	Fernández de Bobadilla, G. (1954)	Castellón (CS)
22	A-20	Turruntés	01/01/03	Albillo	García de los Salmones, N. (1914)	Logroño (LO)
23	L-31	Temprano	01/29/73	Palomino / Albillo	Clemente, S. (1807)	Valladolid (VA)
24	L-27	Temprano Blanco	01/29/73	Palomino / Albillo	Clemente, S. (1807)	Valladolid (VA)
25	K-31	Malvasía	01/10/75	Malvasía / Albillo	Cabello, F. (1992)	León (LE)
26	F-30	Albarín Blanco	02/15/71	Albillo	Janini, R. (1922)	Oviedo (O)
27	K-38	Albillo Mayor	01/01/42	Turruntés	García de los Salmones, N. (1914)	Palencia (P)
28	O-23	Castellano	02/15/74	Mantúo / Albillo	Fernández de Bobadilla, G. (1954)	Castellón (CS)

race of the Henares River. The soil has a loamy texture with high levels of active calcium carbonate, being classified as belonging to the order "Alfisol" within the group "haploxeralfs" according to USDA edaphilogical classification. The average rainfall is 469 annual mm and the average temperature is 13.1ºC, corresponding to a dry Mediterranean climate. The plantation is 35 years old and contains four plants of each accession. Grapevines are pruned in vessel, with three branches and 8 to 10 buds per vine.

Ampelographic descriptions. Morphological characterizations were performed following the descriptor list for the distinction of genus and varieties of the OIV [26]. Descriptors used are listed in Table 2. This description was performed for two consecutive years by three different ampelographers. As a result, a total of six independent measurements were obtained for each descriptor.

Isozyme analysis. Isozyme analyses were performed on wood samples collected in October after leaf fall and were frozen and maintained at -20°C [7]. Tissue extracts were performed following the protocol of Altube et al. [2]. The isozymes analyzed were catechol oxidase (CO) E.C.1.10.3.1., acid phosphatase (ACPH) E.C. 3.1.3.2., glutamate oxaloacetate transaminase (GOT) E.C.2.6.1.1., and superoxide dismutase (SOD) E.C.1.15.1.1. Gel staining for CO and ACPH followed the procedure of Schwennesen et al. [30], whereas for GOT and SOD, the procedures of Sánchez-Yélamo [29] and Baum and Scandalios [3] were followed, respectively. For each accession, the presence or absence of every isozyme band in each isozyme system was scored visually by two different persons and binary coded (as 1 or 0).

AFLP analysis. Total genomic DNA was isolated from young frozen leaves according to the procedure described by Dellaporta et al. [12]. Extraction buffer was supplemented with 1% polyvinylpyrrolidone in order to eliminate polyphenols [20]. AFLP analysis was performed following the protocol of Keygene N.V. [37], with slight modifications described by Cervera et al. [8]. Preamplification was carried out using *Eco*RI + A / *Mse*I +

Table 2 Ampelographic descriptors used in this analysis and modal values for 28 Albillo accessions and associated synonyms using a total of six independent measurements for each of the 43 ampelographic descriptors.

		Cultivars																										
	Ampelographic descriptors	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
002	Young shoot: Distribution of anthocyanin coloration of tip	3	2	2	3	3	3	3	3	3	3	2	3	2	2	3	3	3	3	3	3	3	3	2	2	2	2	1	2
003	Young shoot: Intensity of anthocyanin coloration of tip	5	8	7	7	6	5	6	6	6	5	5	5	5	6	7	5	6	6	6	6	7	7	5	6	7	3	1	5
004	Young shoot: Density of prostrate hairs of tip	5	2	6	7	7	7	7	7	7	6	5	6	2	2	5	5	6	6	7	7	7	6	3	3	2	7	4	3
007	Shoot: Color of dorsal side of internodes	2	2	2	1	2	3	1	2	2	2	2	1	2	2	3	2	2	1	2	1	1	2	2	2	2	2	2	2
008	Shoot: Color of ventral side of internodes	2	2	2	1	1	2	1	1	1	2	2	1	2	2	3	2	2	1	1	1	1	2	1	2	2	2	2	1
015	Shoot: Anthocyanin coloration of bud scales	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1
017	Tendrils: Length	3	4	1	5	4	5	4	3	3	3	5	5	7	4	5	3	5	3	4	4	1	4	3	3	5	2	4	3
051	Young leaf: Color of the upper side	3	4	4	3	3	3	3	2	3	4	3	3	4	3	4	3	3	3	3	3	3	4	4	4	4	3	3	3
053	Young leaf: Density of prostrate hairs between veins at lower side of leaf	8	1	8	7	8	7	7	7	8	6	5	7	3	1	7	6	6	7	8	7	8	7	3	3	3	9	5	8
067	Mature leaf: Shape of blade	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	1	4
068	Mature leaf: Number of lobes	3	4	3	4	3	3	3	3	3	4	4	3	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	3
070	Mature leaf: Anthocyanin coloration of main veins on upper side of blade	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	2	1	1	1	1	3	1	1	1	1	3	3
072	Mature leaf: Goffering of blade	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	2	2	1	1	1	1	1	2
074	Mature leaf: Profile	1	1	1	1	1	1	1	1	4	1	1	1	1	5	1	5	5	1	5	3	1	1	4	1	1	1	1	1
075	Mature leaf: Blistering of upper side	4	5	4	3	4	5	5	4	4	3	3	4	4	4	4	4	4	3	4	4	6	4	5	5	4	7	3	5
076	Mature leaf: Shape of teeth	3	3	3	2	4	3	3	3	2	2	4	3	3	2	3	2	2	3	3	3	3	2	3	3	3	3	2	3
079	Mature leaf: General shape of petiole sinus	2	2	2	2	2	2	2	2	2	2	3	2	3	2	3	3	2	2	2	2	4	3	3	3	3	2	3	2
080	Mature leaf: Shape of base of petiole sinus	3	3	3	1	3	2	2	2	1	2	3	2	3	1	3	3	1	1	3	1	3	2	3	3	3	3	1	2
081-1	Mature leaf: Particularities of petiole sinus	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
081-2	Mature leaf: Naked petiole sinus	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
082	Mature leaf: Degree of opening of upper leaf sinuses	3	4	2	4	3	2	2	4	4	4	4	4	3	4	4	4	4	1	4	3	3	4	3	2	3	2	4	3
083-1	Mature leaf: Shape of base of upper leaf sinuses	2	2	1	2	3	1	2	1	2	2	2	2	2	3	2	2	2	3	3	3	3	2	2	2	2	3	2	3
083-2	Mature leaf: Presence of teeth at base of upper leaf sinuses	1	1	1	2	1	2	1	1	1	2	2	1	1	1	1	2	2	1	1	1	1	1	1	1	1	2	2	1
084	Mature leaf: Density of prostrate hairs between veins (lower side)	5	1	6	3	4	5	5	5	5	4	5	5	1	1	3	5	5	3	3	7	7	7	1	1	1	5	5	3
087	Mature leaf: Density of erect hairs on main veins (lower side)	4	5	1	5	1	3	1	1	1	1	3	1	3	3	5	1	5	1	1	1	1	1	4	3	6	1	3	3
090	Mature leaf: Density of prostrate hairs on petiole	1	1	1	2	1	5	1	5	1	3	3	1	1	1	1	3	3	1	2	5	1	1	1	1	1	1	1	1
091	Mature leaf: Density of erect hairs on petiole	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	3	1	1	1
202	Bunch: Size	2	3	3	5	2	3	1	2	1	1	1	2	4	3	2	3	4	1	3	6	8	4	4	3	2	2	3	3
203	Bunch: Length	2	4	3	4	3	4	2	3	3	2	3	2	4	4	3	2	3	2	2	4	7	3	3	3	3	2	3	7
204	Bunch: Density	4	7	8	3	5	9	6	6	5	6	6	5	5	3	7	5	6	5	3	4	5	7	5	5	5	8	3	7
206	Bunch: Length of peduncle	1	1	1	2	1	1	1	1	1	1	1	1	2	1	3	3	4	1	2	3	3	2	2	3	1	1	1	1
207	Bunch: Lignification of peduncle	5	1	1	1	5	5	1	1	5	1	1	1	1	1	1	1	1	1	1	5	1	7	1	1	1	5	1	1
208	Bunch: Shape	1	1	1	2	1	1	1	1	3	1	2	1	3	1	1	2	2	1	1	3	2	1	2	1	1	1	2	2
209	Bunch: Presence of a wing	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	3	3	3	2	3	4	2	3	2	2	2	2	4
220	Berry: Size	3	5	5	5	3	5	5	5	5	3	3	5	5	5	5	3	5	1	3	5	5	5	5	5	5	5	3	5
221	Berry: Length	7	5	7	5	5	7	7	7	7	5	5	7	7	7	7	3	7	1	3	7	7	7	7	7	7	7	5	7
223	Berry: Shape	2	2	6	2	2	6	2	2	3	2	2	2	2	2	2	2	2	1	2	2	2	6	2	2	2	3	3	3
225-1	Berry: Color of skin	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
301	Time of bud burst	1	1	2	2	1	1	4	1	1	9	9	3	1	2	1	5	8	3	3	5	5	5	1	1	1	3	1	3
503	Berry: Single berry weight	3	3	3	2	1	3	3	3	2	2	2	2	3	3	2	1	2	1	2	3	3	3	3	3	3	2	2	3

C primers. Two primer combinations were used for selective amplification: 2 *Eco*RI (+ ACC, + ACT) / *Mse*I + CAT and 2 *Eco*RI (+ ACC, + ACT) / *Mse*I + CTG. Only polymorphic scorable bands were considered in this analysis. The presence or absence of these bands was scored visually by two different persons and binary coded (as 1 or 0).

Statistical analysis. The statistical analysis was carried out with the help of the program Numerical Taxonomy System (NTSYS v.2.02g) [28]. For every marker used, a matrix was built containing all the values for each specific accession. Data matrices were used to calculate distance or similarity matrices using different indexes depending on the nature of the data. For ampelographic data, a distance matrix was constructed from the correlation distance index. When using isozyme and AFLP data, estimates of genetic similarity (GS) between pairs were based on the number of shared bands according to Dice [33] [GS(ij) = 2a / (2a + 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]. All matrices were subjected to cluster analysis by the unweighted pair-group method analysis (UPGMA) and

dendrograms were constructed from clustering results obtained with the TREE program. Good-fit of cluster analysis (between the similarity matrix and the dendrogram obtained) was measured by calculating the cophenetic correlation between the similarity matrix and the cophenetic matrix for each analysis. Matrix comparisons were made to determine the agreement between isozyme and AFLP-based similarity matrices using the Mantel test [22].

Results

Morphological characterization of Albillo accessions. Twenty-eight accessions identified under the name of Albillo or cited as a synonym of this variety (Table 1) were morphologically characterized using 43 descriptors [26]. The modal values for these descriptors are presented in Table 2. Using the correlation coefficient, we estimated the phenetic distances between every pair of accessions and these were represented in a dendrogram using UPGMA method (Figure 1). Three different clusters could be identified at similarity level of 0.7. The first cluster from Nieves Temprano (Z) to Blanco Castellano (CS) included a total of 14 accessions, where Albillo (M) and Albillo (LE) were highly similar. The second cluster, from Albillo (BU) to Albillo (ZA) included six accessions, with Blanco del País (BU) and Albillo (BU) being the most related. Finally, the third cluster included a total of seven accessions from Albillo (O) to Castellana Blanca (SG). Within this cluster Albillo (O) was highly related to Malvasía (LE) and Temprano (VA) was highly related to Temprano Blanco (VA). These data suggested the existence of three different groups of Albillo and supported some of the previously defined synonymies such as Albillo (O) and Malvasía (LE) as well as Albillo (BU) and Blanco del País (BU). However, results showed a higher phenetic distance between some accessions within each of these clusters than would be expected for synonym varieties. With the aim to solve this problem, the genetic distances among accessions were analyzed using two types of molecular markers: isozymes and AFLPs.

Isozyme phenotypes distinguished four groups of Albillo. Four different isozyme systems, CO, ACPH, GOT, and SOD, were used to analyze the accessions. Isozyme profiles could be organized in specific patterns (Figure 2A) and the profiles shown by every accession are displayed in Figure 2B. The cophenetic correlation between the similarity and the cophenetic matrices was high (0.89, $p = 0.002$) indicating a good fit of cluster analysis. As shown in Figure 2B, the dendrogram suggests the existence of four main groups of accessions. The first cluster, characterized by isozyme phenotypes G for ACPH, O for CO, A for GOT, and A for SOD, includes accessions known as Nieves Temprano (Z), Albillo (AV, M, LE, and ZA), Temprano de Campo Real (M), Temprano de Mora (TO), and Albillo de Toro (ZA). The second cluster, characterized by isozyme phenotypes C for ACPH, O for CO, A for GOT, and B for SOD, includes accessions known as Albillo (O and SA), Malvasía (LE), Temprano (VA), and Temprano Blanco (VA). The third cluster, characterized by isozyme phenotypes I for ACPH, O for CO, A for GOT, and A for SOD, includes accessions known as Albilla

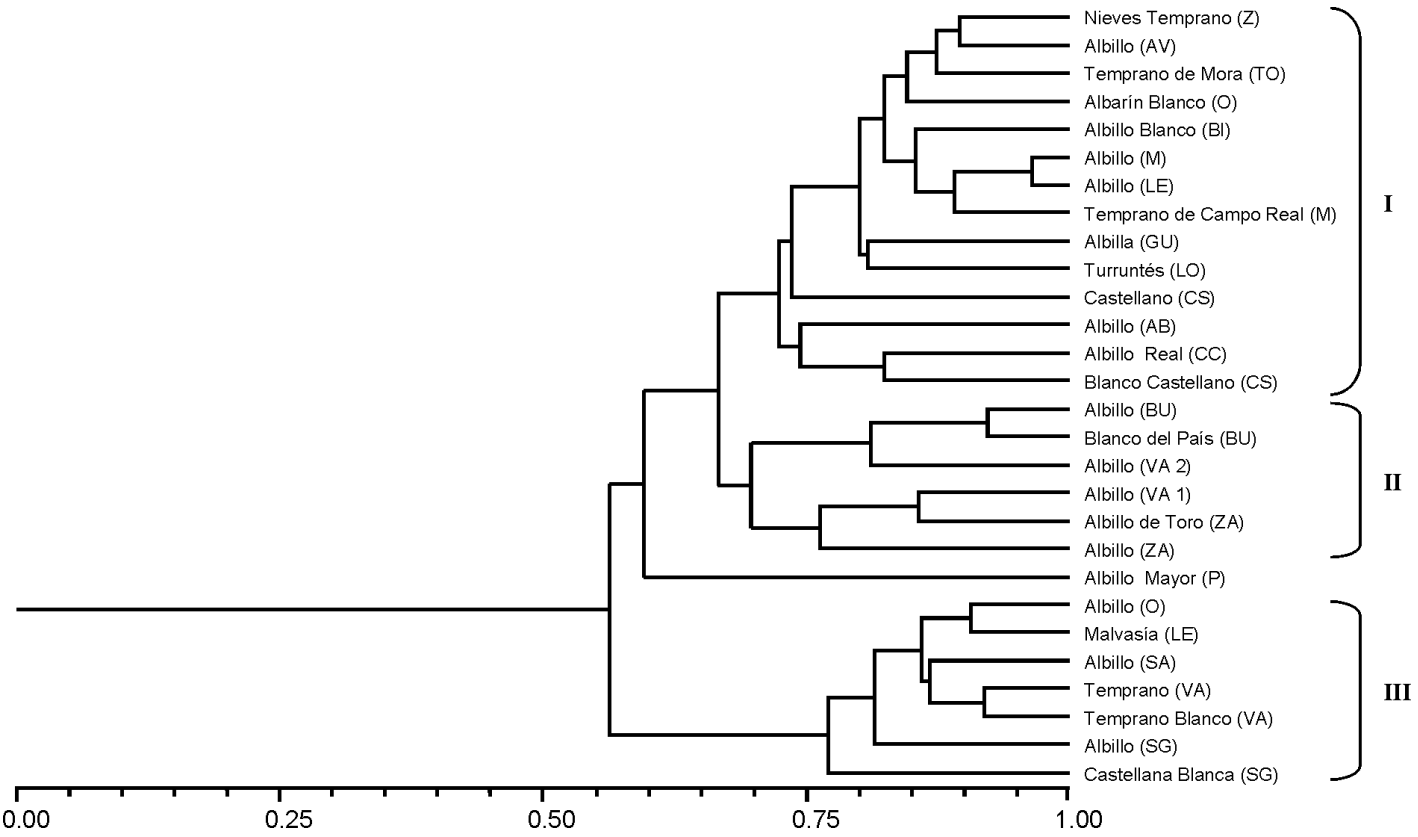

Figure 1 Dendrogram representing morphological relationships among Albillo accessions and associated synonyms (constructed from the ampelographic data distance matrix using the UPGMA clustering method).

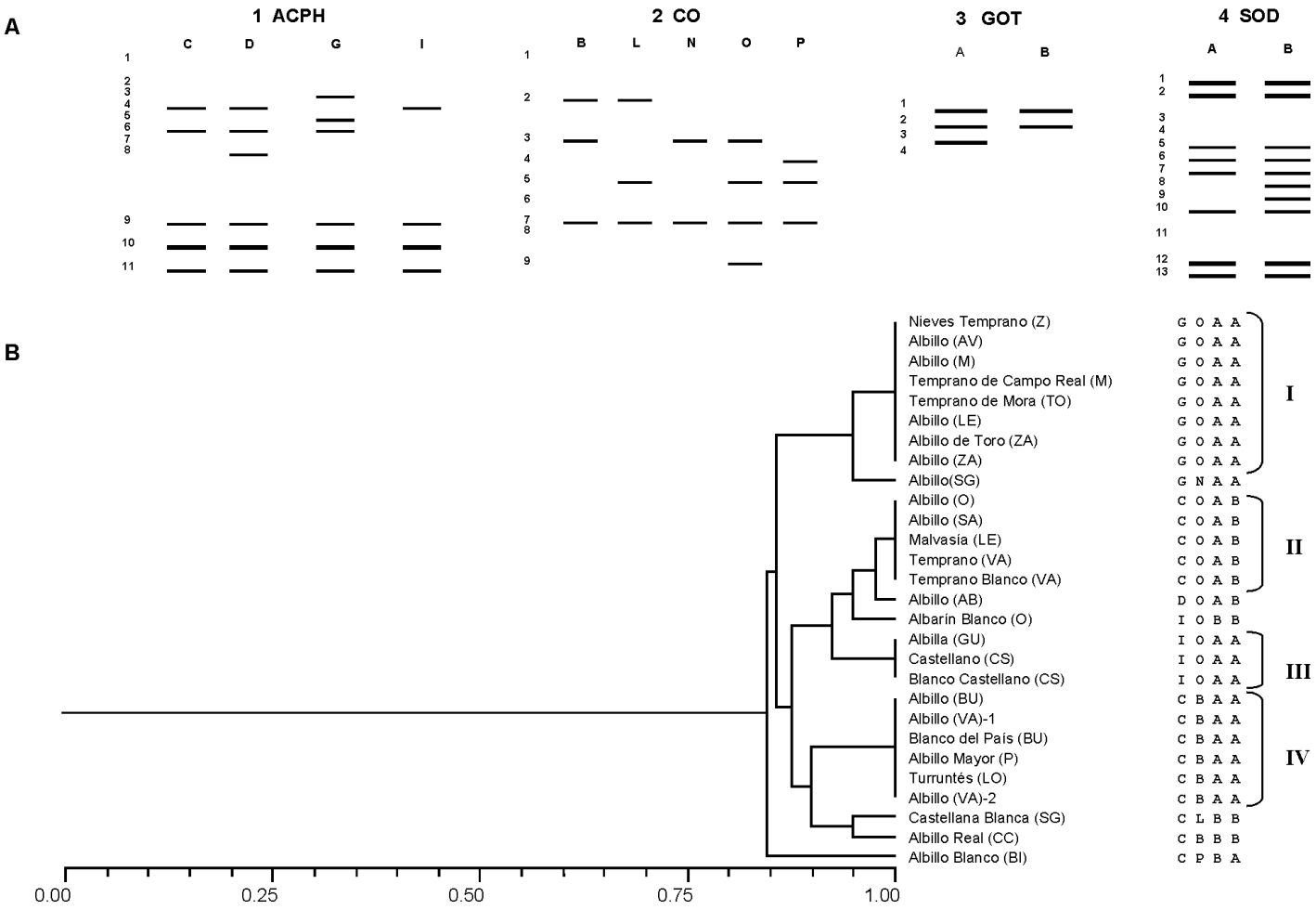

Figure 2 Isozyme analysis of Albillo accessions and associated synonyms. **A**: CO, ACPH, GOT, and SOD isozyme patterns. **B**: constructed using UPGMA clustering method on Dice estimates of genetic similarities based on isozyme data.

(GU), Castellano (CS), and Blanco Castellano (CS). Finally, a fourth cluster is characterized by isozyme phenotypes C for ACPH, B for CO, A for GOT, and A for SOD. It includes accessions Albillo (BU, VA-1, and VA-2), Blanco del País (BU), Albillo Mayor (P), and Turruntés (LO).

Other accessions were distinguished from these main groups but remained related to some of them. That was the case for Albillo (SG), which was closely related to the first cluster but differed in the CO phenotype; Albillo (AB) and Albarín Blanco (O) were related to the second cluster but differed in their ACPH and ACPH/GOT phenotypes, respectively. Finally, other accessions, like Castellana Blanca (SG), Albillo Real (CC), and Albillo Blanco (BI), did not correspond to any of the previously defined clusters and could be considered as false synonymies or homonymies.

The clusters obtained with isozyme data were different from those obtained with morphological data. The first isozyme cluster groups accessions that are morphologically separated in two different groups: Accessions Nieves Temprano (Z), Albillo (AV, M, and LE), Temprano de Campo Real (M), and Temprano de Mora (TO) (cluster I) and Albillo de Toro (ZA) and Albillo (ZA) (cluster II). The second isozyme cluster is coincident with the third morphological cluster, although this morphological cluster includes additional accessions such as Albillo (SG) and Castellana Blanca (SG). The third isozyme cluster is included within the first morphological cluster, indicating that this cluster is heterogeneous. Finally, the fourth isozyme cluster corresponds to part of the second morphological cluster, although morphological analysis includes Turruntés (LO) accession within the first cluster. In order to solve the discrepancies found between both methods and to analyze genetic variability within the isozyme clusters, we carried out an AFLP analysis.

AFLP analysis of Albillo. Two different primer combinations were used for genetic characterization of the 28 accessions. In addition, accession Richter 110, corresponding to a hybrid (*Vitis berlandieri* x *V. rupestris*), was used as a representative outgroup in the analysis and serves as comparison with previous analysis [8,9]. Selection of primer combinations [2 E (+ACC, + ACT) / M + CAT and 2 E (+ ACC, + ACT) / M + CTG] was based on previous work and allowed identification of 107 and 92 total bands respectively, ranging from 70 to 700 nucleotides in samples analyzed. Twenty-two and 13 nonscorable bands obtained using these two primer combinations were excluded

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

Primer combination	Total bands	Polymorphic scorable bands	Total polymorphic bands[a]	% polymorphic scorable bands	% total polymorphic bands[a]
2 E (+ ACC, + ACT) / M + CAT	107	62	84	57.9	78.5
2 E (+ ACC, + ACT) / M + CTG	92	48	61	52.2	66.3
Average	199	110	145	55.3	72.9

[a]Scorable and nonscorable polymorphic bands.

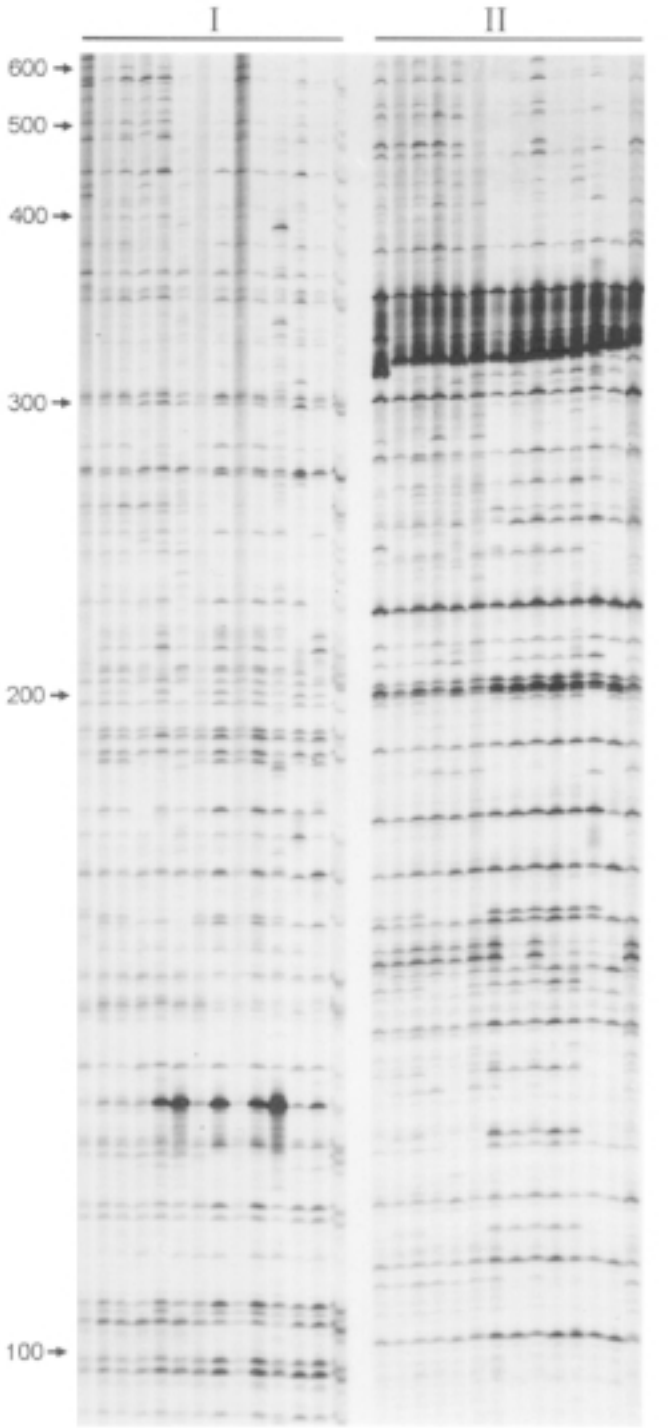

Figure 3 AFLP analysis of Albillo accessions. DNA fingerprints were generated using the primer combination 2 EcoRI (+ ACC, + ACT) / MseI + CAT (I) and 2 EcoRI (+ ACC, + ACT) / MseI + CTG (II). Arrows indicate size marker positions.

from the study. Sixty-two and 48 bands showing clear scorable polymorphism representing 55% of total bands on average (Table 3) were scored for their presence or absence. AFLP patterns obtained for some accessions using both primer combinations are shown in Figure 3 and were repeatedly found in different experiments using different DNA extractions and plants belonging to the same accession.

A dendrogram illustrating genetic similarity values obtained from AFLP data is displayed in Figure 4. The cophenetic correlation between the similarity matrix and the cophenetic matrix was high (0.95, $p = 0.002$) indicating a good fit of cluster analysis. Most of the accessions showed GS levels ranging between 0.48 and 0.98. Based on previous results [8], accessions showing similarities higher than 0.90 could be considered as accessions belonging to the same variety, while accessions typically considered as different varieties show similarities ranging from 0.6 to 0.90. Moreover, accessions belonging to varieties with close genetic relationships (parents and offspring or siblings derived from the same cross) show values of genetic similarity ranging from 0.8 to 0.9 [9].

The dendrogram displayed in Figure 4 suggests the existence of three main groups of accessions. The first cluster groups accessions Albillo (VA-1 and VA-2), Blanco del País (BU), Albillo Mayor (P), and Turruntés (LO). The second cluster includes accessions Albillo (SA and O), Temprano Blanco (VA), Temprano (VA), and Malvasía (LE). No polymorphism at the DNA level was detected between Temprano (VA) and Malvasia (LE). Finally, the third cluster includes accessions Albillo (LE, AV, M, and ZA), Albillo de Toro (ZA), Temprano de Campo Real (M), Temprano de Mora (TO), and Nieves Temprano (Z). No polymorphisms were detected between Albillo (LE) and Albillo (AV) as well as between Temprano de Campo Real (M) and Albillo (ZA).

There were several oddities to this classification regarding the previous ones based on morphological and isozyme data. Based on AFLPs, accession Albillo (BU), which represents an accession closely related to the first cluster based on ampelographic descriptions as well as isozyme data, could represent the result of the hybridization between an accession of this group and a different variety [GS ≥ 0.78 when compared to Albillo (VA-1) and (VA-2)]. Additionally, based on AFLP similarity data, Albarín Blanco (O) may be related to the second and third cluster showing genetic similarity ranging from 0.78 to 0.81 and 0.75 to 0.79, respectively. Finally, Albillo (SG) could represent an accession closely related to the third cluster based on ampelographic descriptions as well as isozyme data. AFLP data suggests that this accession, showing GS values ranging from

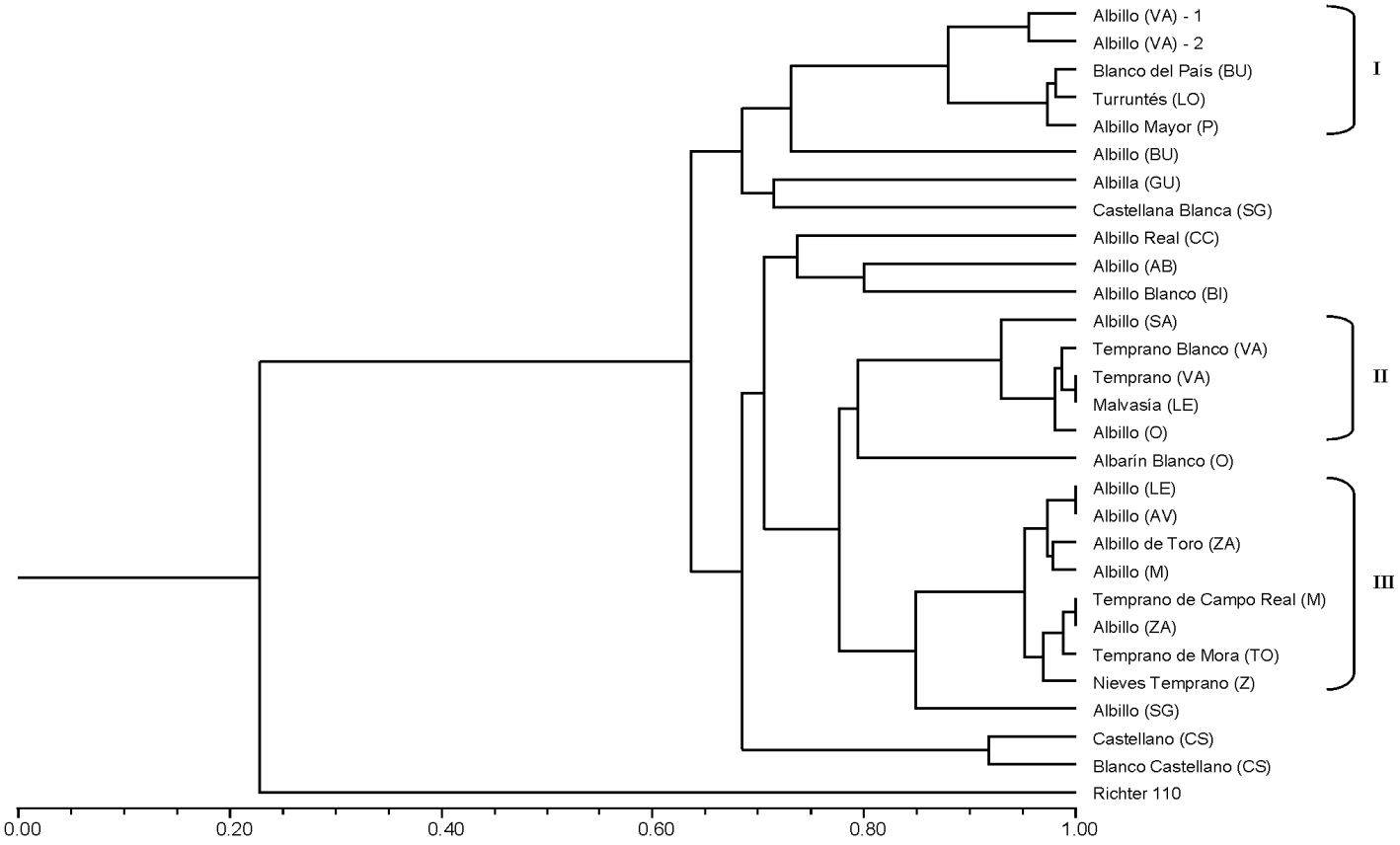

Figure 4 Dendrogram representing the genetic similarities among Albillo accessions and associated synonyms (constructed using UPGMA clustering method on Dice estimates of genetic similarities based on AFLP data).

0.81 to 0.87, could be the result of the hybridization between an accession of this cluster and a different variety. Furthermore, accessions grouped within the second cluster such as Albillo (SA and O), Temprano Blanco (VA), Temprano (VA), and Malvasía (LE) are more related to the third cluster than to the first.

Other accessions, such as Albilla (GU), Albillo (AB), Albillo Real (CC), and Albillo Blanco (BI), were identified as homonyms based on the low genetic similarity observed when compared to other Albillo accessions (GS < 0.7). These accessions can be described as belonging to different varieties. This analysis also allowed identification of false synonyms such as the related accessions Castellano (CS) and Blanco Castellano (CS) or Castellana Blanca (SG).

The statistical significance of the correlation between isozyme and AFLP-based similarity matrices was verified with the Mantel permutation test [22]. Although the results of the test were in the lowest level of correlation that can be considered statistically significant (0.61, $p = 0.002$), we can explain these results based on the differences of the marker technology used and the reduced number of isozyme markers analyzed.

Discussion

Varietal groups of Albillo. All three approaches used to characterize accessions under the name Albillo and their synonyms allowed identification of at least three different varietal groups of Albillo, consistent with geographical and historical origin, and which may represent three homonym varieties (Figure 5). As Albillo de Madrid was the first variety described under this variety [1], and it shows the closest phenotype to the given description, we named the first group after this variety. This

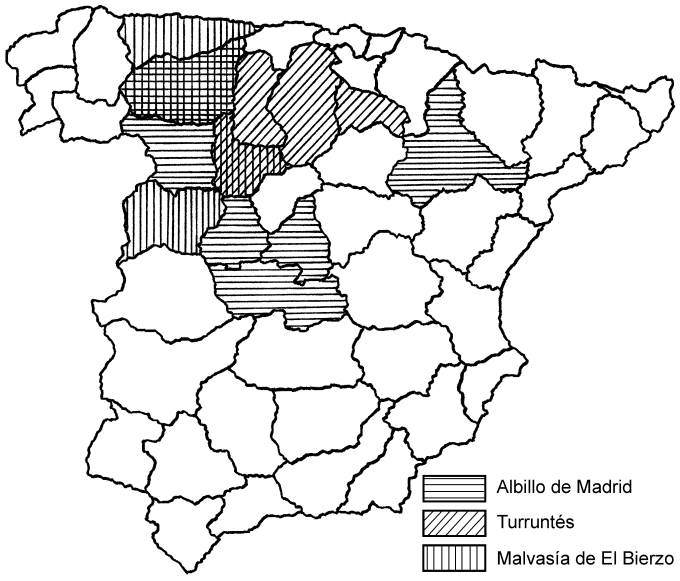

Figure 5 Growing areas in Spain for the Albillo varieties identified.

group, recognized by the DO Vinos de Madrid, would include accessions Albillo (M, LE, AV, and ZA) and the synonymous accessions Albillo de Toro (ZA), Temprano de Campo Real (M), Temprano de Mora (TO), and Nieves Temprano (Z). The second group includes Albillo accessions recognized by the DO Ribera del Duero [Valladolid (VA-1) and (VA-2)] and their synonym accessions Blanco del País (BU), Albillo Mayor (P), and Turruntés (LO). We believe that these accessions correspond to the variety known as Turruntés, first described by Manso de Zúñiga [21], and therefore propose the name of Turruntés for this variety. The third group of Albillo would include most of the Albillo accessions from northwestern Spain [Salamanca (SA) and Oviedo (O)] with Malvasía (LE) accession recognized by the DO Bierzo. We therefore propose the name of Malvasía de El Bierzo for this group of accessions to distinguish them from the other Malvasía varieties. Synonyms for this group are the accessions Temprano (VA) and Temprano Blanco (VA), previously classified as Palomino. Geographical distribution of these three Albillo varieties is indicated in Figure 5. Accessions belonging to the proposed groups showed GS values higher than 0.9, suggesting a monophyletic origin and genetic divergence based on the accumulation of somatic mutations along vegetative propagation. Each group could be considered as a varietal "sortogroup" when the concept developed by Negrul [25] is applied.

Our classification does not include other homonym accessions such as Albillo (AB), Albilla (GU), Albillo Blanco (BI), and Albillo Real (CC) or the incorrect synonyms of Castellana Blanca (SG). Additionally, grouped accessions Castellano (CS) and Blanco Castellano (CS) have been incorrectly cited as Albillo synonyms by Fernández de Bobadilla [14]. Albillo (BU), which falls both morphologically and with isozyme analysis within the group of Turruntés, is excluded from this group by the results of AFLP analysis. Based on AFLP data, Albillo (BU), Albarín Blanco (O) (synonym cited by Janini [18]), and Albillo (SG) accessions are genetically related to Turruntés, Malvasía de El Bierzo/Albillo de Madrid, and Albillo de Madrid varieties, although they do not share with them a monophyletic origin. These accessions could only be considered members of the corresponding varieties if the concept of variety-population (plants that share morphological characteristics) is applied. Further analysis using co-dominant markers will be required to understand the genetic relationships among Albillo (BU), Albarín Blanco (O), and Albillo (SG) and their most related clusters.

Applications of different methodologies. When comparing the three different methodologies, isozymes and AFLPs gave the most consistent results, distinguishing the same groups. Morphological analyses yielded clusters that did not completely account for the genetic similarity found among the accessions when using molecular markers. Morphological descriptions may be confounded with environmental variation and be prone to subjective evaluations. As homonyms or incorrect synonyms are based on morphological and agronomical similarities, morphological descriptors are less valid to solve these uncertainties. For example, Albillo Blanco (BI) and Albarín Blanco (O), with agronomical and morphological features similar to those of Albillo de Madrid and closely related on the basis of the morphological descriptors, are not genetically related based on molecular analyses. On the other hand, morphological descriptors are required to establish the link between historic descriptions and the molecular fingerprints revealed by isozyme or DNA-based molecular markers such as AFLPs. In this way, the identification of Albillo de Madrid, five hundred years after its first description [1] is only possible on the basis of the ampelographic analysis.

Grapevine variety characterization can be very complex, yet it may be positively managed through the use of integrated approaches including complementary techniques. While morphological description should be the starting point of characterization, it is not always possible to perform an accurate analysis. This study shows that isozymes are still a useful tool for first-hand high-volume varietal identification. The use of additional isozyme systems could still provide higher resolving power when required. Isozymes, however, are not the right markers to search for intravarietal variation or to distinguish different varieties belonging to the same sortogroup, which could only be solved by DNA markers such as AFLPs.

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