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Molecular and ampelographic characterisation of *Vitis vinifera* L. 'Albariño', 'Savagnin Blanc' and 'Caíño Blanco' shows that they are different cultivars

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Abstract

The grapevine cultivar 'Albariño' is one of the oldest grown in the vine-growing areas of north-western Spain and northern Portugal. Since recognition of Origin Denomination status for the Rías Baixas region (the coast of western Galicia, Spain) in 1987, the economic importance of this cultivar has increased, and its grapes are now among the most expensive in Spain. The area occupied by 'Albariño' vines in this region is increasing every year, and the wines made from its grapes are gaining international recognition. These events, plus the fact that 'Albariño' was little known outside its traditional growing area, have led to speculation about its origin and the existence of synonyms. Misnames of 'Albariño' have included 'Savagnin Blanc' and 'Caíño Blanco'. The present work compares 'Albariño', 'Savagnin Blanc' and 'Caíño Blanco' ampelographically (i.e., it compares shoot, leaf, grape cluster, berry and seed characteristics) and molecularly using microsatellite markers. The results show that they are in fact three different cultivars providing a complete description. For 'Caíño Blanco', there is little previously reported information.

Additional key words: identification, grapevine, microsatellites, synonymy.

Resumen

La caracterización molecular y ampelográfica de clones de 'Albariño', 'Savagnin Blanc' y 'Caiño Blanco' (*Vitis vinifera* L.) demuestra que son cultivares diferentes

El 'Albariño' es uno de los cultivares más antiguos de la zona vitícola del Noroeste de España y norte de Portugal. Desde la aprobación en el año 1987 de la Denominación de Origen Rías Baixas, este cultivar es el de mayor valor económico de esta zona vitícola, y su uva alcanza uno de los precios más elevados de España. Su superficie de cultivo, dentro de esta Denominación de Origen, se multiplica año tras año, y sus vinos adquieren fama internacional. Todo ello, unido a que era un cultivar poco conocido fuera de esta pequeña zona vitícola española, ha llevado a la aparición en los últimos años de diversas especulaciones sobre su origen, y a la atribución de diferentes sinonimias. Entre los nombres erróneamente atribuidos al 'Albariño' se encuentran 'Savagnin Blanc' y 'Caíño Blanco'. En el presente trabajo se hace un estudio comparativo de los tres cultivares a nivel ampelográfico (brote, hoja, racimo, baya, semilla) y a nivel molecular por medio de marcadores microsatélite. Los resultados demuestran que 'Albariño', 'Savagnin Blanc' y 'Caíño Blanco' son cultivares distintos, proporcionando una completa descripción de cada uno. De uno de ellos, 'Caíño Blanco', existe muy poca información previa.

Palabras clave adicionales: identificación, microsatélites, sinonimias, vid.

Introduction

The grape cultivar 'Albariño' is one of the oldest grown in the vine-growing area of north-western Spain (Huetz de Lemps, 1967). In 1987, the status of Origin Denomination was granted to the Rías Baixas area of western Galicia - and the cultivar 'Albariño' is the most important grape cultivar used in manufacture of its wines. Until now, this cultivar was grown solely for personal consumption, but since then the area of its production has increased enormously (Consejo Regulador de la Denominación de Origen Rías Baixas, 2004). It is now one of the most expensive grapes in the country. In Galicia, its only known synonym is 'Abelleiro', which is used in the Rosal area. In Portugal it is known as 'Alvarinho',

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'Galego' or 'Galeguinho' (Lara, 1993; Freijanes and Alonso, 1997). There are several hypotheses surrounding the origin of 'Albariño' (Posada, 1978; Johnson, 1990; Del Caño Abad, 1991; Hidalgo, 1993). However, in Galicia a relatively large number of 200-300 year-old 'Albariño' vines are known, and the geographical characteristics of Galicia and the presence in several parts of northern Spain of specimens of *Vitis vinifera silvestris* (Rivera and Walker, 1989; Martínez de Toda, 1991) —the ancestor of *V. vinifera sativa* (the cultivated vine)— suggest that 'Albariño' could well be a native of the north-western Iberian Peninsula.

The oldest description of this cultivar is that of Casares (1843). At the National Wine Exhibition in 1877, 'Albariño' was included as a variety specifically cultivated in the Galician Provinces of Lugo and Pontevedra (Lara, 1993; Pérez *et al*., 1993). Ministerio de Fomento (1911), García de los Salmones (1915) and Marcilla (1968), reported 'Albariño' as being grown in the Province of Pontevedra, Galicia. A full description of the cultivar was provided by Hidalgo in 1987.

Martínez and Mantilla (1993) and Martínez *et al*. (1994), surveyed the entire Galician vine-growing area, and located and described more than forty 200-300 year-old specimens of 'Albariño' vines. All had the same ampelographic characteristics as 'Albariño' vines in the oldest and newest vineyards. All growers in the region, without exception, recognised the typical 'Albariño' cultivar characteristics in these plants. Their identity was confirmed by molecular studies of six microsatellite loci (Loureiro *et al*., 1998).

Part of the confusion surrounding the origin of the 'Albariño' cultivar lays in the fact that plant material erroneously identified as 'Albariño' was sent from Galicia to different Spanish grape collections and was later identified by Loureiro *et al*. (1998) as 'Savagnin Blanc'. However, material from these collections had already been sent to other national and international collections. It is possible that Truel (1983), Imazio *et al*. (2002) and Bourke (2004) used this material, and identified what they thought was 'Albariño' as being synonymous with 'Savagnin Blanc' (White Traminer). This is a well-known traditional grapevine thought to be native of the Tyrolese place Tramin in Italy, and cultivated in many European countries (France, Switzerland, Austria and Germany). This cultivar has been characterised and described by several authors (Foëx, 1891; Galet, 1958; Ambrosi *et al*., 1994; Boidron *et al*., 1995). In Spain its cultivation is very limited. 'Savagnin Blanc' was introduced into the Principality of Asturias (northern Spain) after the Spanish phyloxera epidemic in the late 19th century (Martínez and Pérez, 2000). Over all of this period, 'Savagnin Blanc' has been confused with 'Albarín Blanco' (Blanco Verdín), a traditional grape cultivar which is grown in Asturias (Santiago *et al*., 2005).

On other occasions, 'Albariño' has been confused with 'Caíño Blanco' (Cincinato da Costa, 1900; Loureiro *et al*., 1998). 'Caíño Blanco' is cultivated in the Rosal area, which is part of the Rías Baixas Origin Denomination. This cultivar is included in the Spanish provisional list of vine varities of commercial interest. However, there is little previous information on this cultivar. In Portugal, 'Caíño Blanco' (Cainho de Moreira) is known as 'Alvarinhão' or 'Alvarinho Espanhol' (Mota and Sá, 1986) and is cultivated in the Vinhos Verdes region of northern Portugal.

The market importance that 'Albariño'-based wines have acquired in recent years has led to increased national and international interest in this cultivar. Concern has often been raised whether 'Albariño' is truly synonymous with 'Savagnin Blanc' and 'Caíño Blanco'.

The aim of this work was to compare the ampelographic and molecular characteristics of the grape cultivar 'Albariño' with those of its potentially synonymous cultivars by providing a complete description of them. This is particularly the case for 'Caíño Blanco' where there have been few previous studies.

Material and Methods

Plant material

The study material comprised 10 specimens of single clones of each 'Albariño', 'Savagnin Blanc' and 'Caíño Blanco' cultivars. The material was from the collection of the *Misión Biológica de Galicia* (*Consejo Superior de Investigaciones Científica*, CSIC), Pontevedra, Spain. All plants were grown in the same plot and were subjected to identical productions systems, cultivation conditions and pruning. The 'Albariño' clone was from a 200-400 year-old plant located in the Salnés area (Rías Baixas Origin Denomination). Previous studies have demonstrated the trueness to type of this clone (Martínez *et al.*, 2005). The 'Caíño Blanco' clone was propagated from a mother plant located in the Rosal area (Rías Baixas Origin Denomination) and the 'Savagnin Blanc' clone was from a vine growing in Cangas del Narcea (Asturias).

Ampelographic characterisation

Different variables were recorded for each of the 10 specimens of each grape cultivar. These variables were selected, based on our experience, because of their discriminate power and objectiveness. All measurements were made at different times during the growth cycle.

When shoots were between 10 and 30 cm, the variables proposed by the OIV (1983) were measured (codes 001, 002, 003, 004, 005). One fruiting shoot was examined per specimen per clone.

Between fruit setting and *veraison*, 11 adult leaves (from node 8 or 9 on a fruiting branch) were collected per clone. These were pressed and the variables measured were those mentioned by the OIV (1983) (codes 067, 068, 069, 070, 071, 076, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089).

The variables proposed by Martínez and Grenan (1999) for reconstruction of an average leaf: The linear distances of both sides (d = right; g = left) were measured as straight lines from the beginning to the end of the veins: L, L1, L2 and L3 are the lengths of the primary veins L, L1 and L2 and the secondary vein L3; L5 is the length between the petiole point and vein L3. Lateral sinuses were also measured (d = right; g = left): S1 = length petiole sinus to upper lateral leaf sinus; S2 = length petiole sinus to lower lateral leaf sinus. The angles (right side: A, a, B, b, G, g, and D; left side: A', a', B', b', G', g', and D') were measured as follows: A and A' = angles between the central vein and the first lateral vein; a and a' = angles between the central vein and L1; B and B' = angles between the first and second right lateral veins; b and b' = angles between the first lateral vein and L2; G and G' = angles between the second lateral vein and the first secondary vein; g and g' = angles between the second lateral vein and L3; D and D' = angles between L5 and the tangent from the tip of the petiole along the side of the leaf. Following the same method, the teeth were counted by sector. In each interval, a number was assigned to every tooth: Arabic if the attached vein was a lower secondary vein, Roman if the attached vein was an upper secondary vein. If the vein of the tooth was tertiary, that tooth was given the number of the previous tooth, to which a letter was added.

In September, grapes were harvested and the following observations were made on 10 clusters per clone (one from each specimen), and on 50 berries and seeds per clone:

— Clusters: the variable proposed by the OIV (1983) – code 204 was measured. Cluster shape was also noted (Martínez and Mantilla, 1993), as was the number of clusters per fruiting branch, the total number of clusters per vine, the weight, length and width of clusters, and the peduncle length.

— Berries: the variables proposed by the OIV (1983) – codes 222, 223, 224, 225, 226, 227, 229, 230, 321, 232, 234, 236, 237, 239 and 240 were measured. Berry weight, length and width, pedicel length, and the number of seeds per berry were also recorded.

— Seeds: weight and length.

Other variables measured included weight of grapes per cluster and per vine, and the weight of wood produced by each vine. The grape yield per hectare was calculated from the weight of the grapes per vine (considering the number of vines and their population in the plot). In addition, a fertility index was calculated as: number of clusters per vine × 100/number of shoot buds per vine.

Microsatellite analysis

The following microsatellite loci were analysed: VVS2 (Thomas and Scott, 1993), VVMD5, VVMD7 (Bowers *et al*., 1996), VVMD27 (Bowers *et al*., 1999), ssrVrZAG62 and ssrVrZAG79 (Sefc *et al*., 1999). These markers were developed as a standard set of microsatellite reference alleles for identification of grape cultivars (This *et al*., 2004). The DNA extraction, quantification, PCR reactions and detection of amplified products were carried out according to Santiago *et al.* (2005). The results of the microsatellite analysis were expressed as allele sizes (number of base pairs).

Results

The three cultivars had very similar shoots (Table 1, parameters OIV 001 to 005). However, there were clear differences in leaf shape (parameter OIV 067; Table 1 and Fig. 1). 'Albariño' leaves were pentagonal whereas 'Caíño Blanco' leaves were between pentagonal and cuneiform, and 'Savagnin Blanc' leaves between pentagonal and round. Adult leaf colour (parameter OIV 069) also varied (Table 1). 'Albariño' leaves were between light and medium green, 'Caíño Blanco' leaves medium green, and 'Savagnin Blanc' leaves were darker. In addition, the veins of 'Albariño' leaves were not pigmented (Table 1, parameter OIV 070 and 071), while

Table 1. Mean values for green shoot and mature leaf OIV (1983) characteristics

	Albariño M (max-min)	Caíño Blanco M (max-min)	Savagnin Blanc M (max-min)
OIV 001	7	7	7
OIV 002	2	2	2
OIV 003	3	4.48 (6-1)	5.33 (6-5)
OIV 004	8.33 (9-7)	8.33 (9-7)	8.33 (9-7)
OIV 005	1	1	1
OIV 067	3	2.9 (3-1)	3.41 (4-3)
OIV 068	2	2	2
OIV 069	3-5	3.83 (5-3)	6.32 (7-3)
OIV 070	1	5.74 (8-5)	3.91 (4-3)
OIV 071	1	6.59 (9-5)	3 (3-3)
OIV 076	3	3.55 (4-3)	2.98 (4-2)
OIV 079	3	2.60 (3-1)	4.25 (7-3)
OIV 080	2	1.48 (2-1)	2
OIV 081	1	1.07 (2-1)	1
OIV 082	1	1	1.05 (2-1)
OIV 083	2	1.98 (2-1.5)	1.98 (2-1.5)
OIV 084	5.8 (5-6)	5.93 (8-4)	4.18 (5-3)
OIV 085	1	6.33 (9-5)	4.14 (7-3)
OIV 086	5	6.50 (8-5)	4.95 (6-4)
OIV 087	1	6.17 (9-4))	4.77 (7-3)
OIV 088	9	7.4 (9-1)	9
OIV 089	1	7.67 (9-1)	1

M: mean. max: maximum value. min: minimum value.

'Caíño Blanco' leaves had strongly pigmented major veins, and 'Savagnin Blanc' leaves were weakly pigmented. All three cultivars had prostrate hairs on the underside of their leaves, both on and between veins (Table 1, parameters OIV 084 and 086). However, they differed in that 'Albariño' had no erect hairs on the underside of the leaf (Table 1, parameters OIV 085 and 087), whereas 'Caíño Blanco' leaves showed a high density and 'Savagnin Blanc' leaves a medium density of erect hairs. Only 'Caíño Blanco' had erect hairs on the upper side of its leaves (Table 1, parameter OIV 089).

With respect to leaf size (Table 2, Fig. 1), 'Albariño' leaves were the largest among the three cultivars and 'Caíño Blanco' leaves the smallest. In addition, 'Caíño Blanco' showed the distinguishing characteristic of having a petiole which was always longer than the main leaf vein. This cultivar could also be distinguished because the angles formed by its major veins (with the main vein) were the smallest. In fact, this cultivar has the smallest angles of all those for which we have constructed an average leaf diagram.

Cultivars differed in terms of cluster compactness (Table 3, parameter OIV 204): 'Albariño' and 'Savagnin Blanc' had medium compact clusters, while 'Caíño Blanco' clusters were loose. In addition, 'Albariño' clusters were conical and had wings while 'Caíño Blanco' clusters were between conical and branching and 'Savagnin Blanc' clusters were cylindrical (Table 3, Fig. 2).

With respect to cluster and berry quantitative variables (Table 4), 'Albariño' and 'Caíño Blanco' had two clusters per fruiting branch, while 'Savagnin Blanc' generally only had one. 'Albariño' had the least clusters per vine, although they were somewhat larger than those of the other two cultivars. 'Caíño Blanco' had the highest number of clusters per vine, though these were the smallest. These clusters also had the longest peduncle and the smallest berries. 'Savagnin Blanc' produced the largest berries. Nevertheless, this cultivar frequently bears some smaller berries in the same cluster (Fig. 2). The majority of 'Albariño' and 'Caíño Blanco' berries contained two seeds, whereas 'Savagnin Blanc' berries only had one. The seed of 'Albariño' and 'Savagnin Blanc' were the same size, and bigger than those of 'Caíño Blanco'.

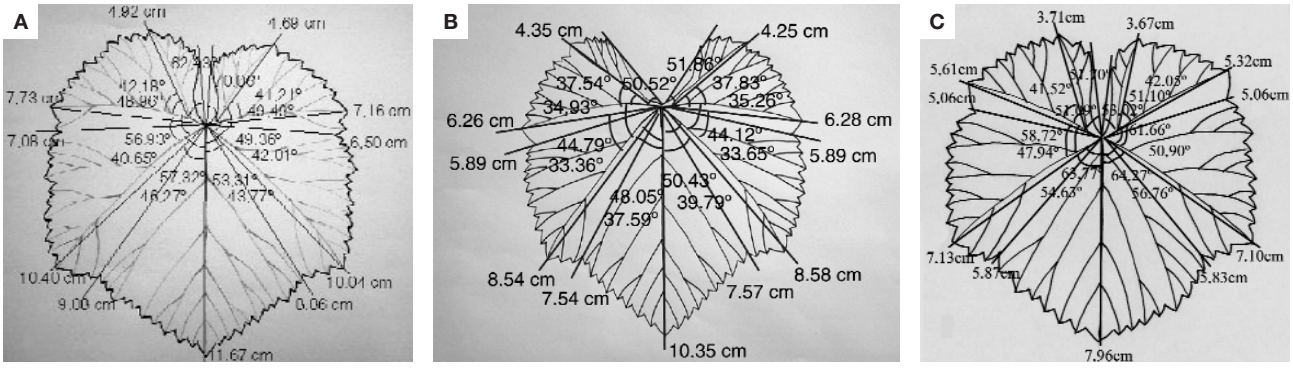

Figure 1. Average leaves of 'Albariño' (A), 'Caíño Blanco' (B) and 'Savagnin Blanc' (C), derived by the method of Martínez and Grenan (1999).

'Albariño' produced almost twice as many kilograms of grapes per vine as the other two cultivars, whose yields were very similar. However, the fertility index (expressed as the number of clusters on each vine with respect to the number of shoots left after pruning), was much higher in 'Caíño Blanco' than in 'Albariño', and even greater than in 'Savagnin Blanc'. 'Albariño' and 'Caíño Blanco' produced similar amounts of wood at pruning, although both produced much less wood than 'Savagnin Blanc'.

At the molecular level, the three cultivars showed different allelic combinations for the 6 microsatellite

Table 2. Mean values and standard deviation (SD) of basic length (cm) and angle (°) variables for «mean leaf» construction

	Albariño		Caíño Blanco		Savagnin Blanc	
	Mean	SD	Mean	SD	Mean	SD
L (cm)	11.67	2.09	10.35	1.25	7.96	0.89
S1d (cm)	8.86	1.34	7.57	1.02	5.83	0.72
L1d (cm)	10.04	1.47	8.58	1.23	7.10	0.77
S2d (cm)	6.50	1.07	5.89	0.90	5.06	0.74
L2d (cm)	7.16	1.17	6.28	0.96	5.62	0.82
L3d (cm)	4.69	0.84	4.25	0.69	3.67	0.66
L3g (cm)	4.92	1.12	4.35	0.74	3.71	0.63
L2g (cm)	7.73	1.54	6.26	0.86	5.61	0.79
S2g (cm)	7.08	1.41	5.89	0.82	5.06	0.75
L1g (cm)	10.40	1.74	8.54	1.17	7.13	0.90
S1i (cm)	9.08	1.52	7.54	0.99	5.87	0.95
L5g (cm)	0.79	0.15	0.51	0.21	0.67	0.12
L5g (cm)	0.76	0.23	0.52	0.24	0.70	0.15
Lp (cm)	10.74	2.54	11.72	2.22	6.91	1.38
A (º)	53.31	7.61	50.43	5.13	64.27	6.44
a (º)	43.77	4.63	39.79	5.51	56.76	5.01
B (º)	49.36	14.04	44.12	3.97	61.66	5.96
b (º)	42.01	8.28	33.65	4.51	50.90	6.64
G (º)	49.49	8.07	37.83	4.82	51.10	6.49
g (º)	41.21	6.28	35.26	3.50	42.05	5.06
G' (º)	48.96	10.28	37.54	5.39	51.09	4.71
g' (º)	42.18	9.21	34.93	6.26	41.52	7.34
B' (º)	56.93	5.15	44.79	4.27	58.72	5.87
b' (º)	40.65	7.58	33.36	5.11	47.94	4.72
A' (º)	57.32	6.53	48.05	5.33	63.77	5.54
a' (º)	46.27	3.49	37.59	4.68	54.63	6.66
D (º)	70.06	16.02	51.86	8.93	53.02	8.94
D' (º)	62.43	11.43	50.52	8.76	51.74	8.06

Table 3. Mean values for clusters, berries and seeds OIV (1983) characteristics in different grape cultivars

	Albariño M (max-min)	Caíño Blanco M (max-min)	Savagnin Blanc M (max-min)
OIV 204	5	3.74 (5-3)	5
Cluster morphology[1]	Conical with wings	Conical and branching	Cylindrical
OIV 222	2	2	2
OIV 223	3	3.11 (3-4)	3.63 (4-3)
OIV 224	2	2	2
OIV 225	1	1	1
OIV 226	2	2	2
OIV 227	3	4.84 (5-3)	5
OIV 229	1	1	1
OIV 230	1	1	1
OIV 231	1	1	1
OIV 232	2	2	2
OIV 234	1	1	1
OIV 236	1	1	1
OIV 237	1	1	1
OIV 239	2	2	2
OIV 240	5	5	5

[1] Martínez and Mantilla (1993).

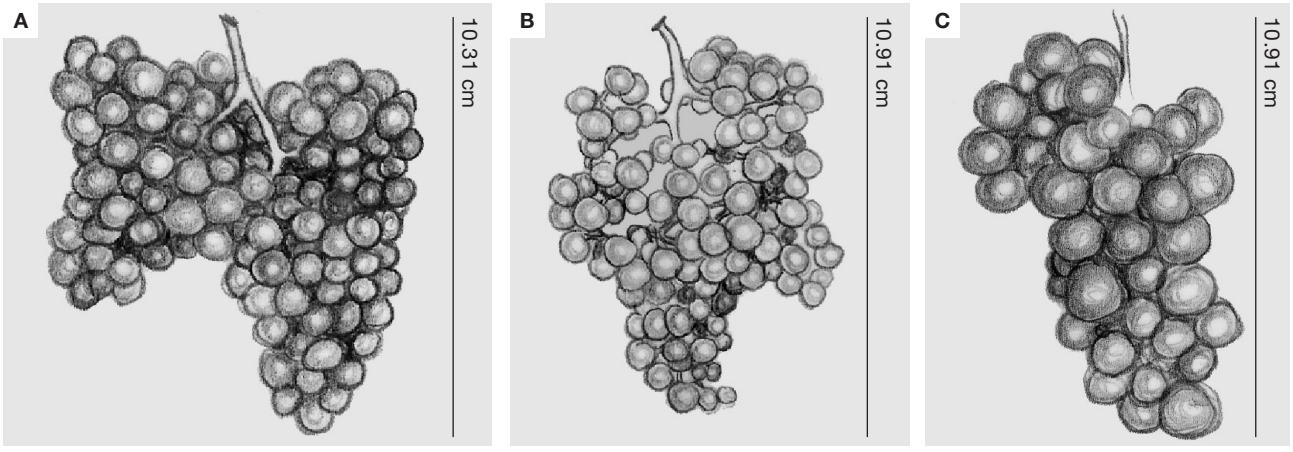

Figure 2. Typical grape clusters with the average length shown in Table 4. A: 'Albariño'. B: 'Caíño Blanco'. C: 'Savagnin Blanc'.

Table 4. Means and standard deviation (SD) for agronomic variables (cluster, berries, seeds and wood pruning)

Variables	Albariño		Caíño Blanco		Savagnin Blanc	
	Mean	SD	Mean	SD	Mean	SD
Clusters per vine shoot	2.11	0.58	2.24	0.54	1.67	0.52
Total clusters per vine	17.22	4.63	35.79	16.15	23.75	1.26
Cluster weight (g)	202.19	66.35	103.74	56.38	196.48	108.12
Cluster length (cm)	10.31	1.23	10.91	2.07	10.91	1.42
Cluster width (cm)	11.46	1.26	7.92	20.9	9.03	1.99
Cluster peduncle length (cm)	1.48	0.48	3.77	2.09	1.56	0.34
Pedicel length (cm)	0.66	0.12	0.53	0.13	0.42	0.08
Berry length (cm)	1.29	0.11	1.23	0.13	1.48	0.11
Berry width (cm)	1.32	0.13	1.20	0.13	1.40	0.08
Berry weight (g)	1.49	0.47	1.19	0.30	1.88	0.36
Number seeds per berry	1.86	1.01	1.85	0.87	1.14	0.35
Seed weight (g)	0.03	0.03	0.02	0.01	0.03	0.01
Seed length (cm)	0.60	0.08	0.54	0.08	0.62	0.06
kg grapes ha^{-1}	7,679.85	3,089.31	4,860.80	5,520.76	4,815.32	5,452.96
kg grapes per vine	3.74	1.51	2.38	2.70	2.36	2.67
Fertility index	9.54	3.74	13.44	4.46	7.49	1.60
Weight of pruned wood (kg)	1.34	0.33	1.41	0.41	0.75	0.38

loci (Table 5). 'Savagnin Blanc' was very different to the other two varieties at two of the loci analysed (VVMD7 and ssrVZAG62). 'Albariño' and 'Caíño Blanco' coincided fully at locus ssrVZAG79, and in one other allele at each of the other loci.

Discussion

'Albariño' growing in the northwest of the Iberian Peninsula has been perfectly identified and the existence of a 200-400 year-old specimens prove 'Albariño' as a traditional cultivar in this region (Martínez *et al*., 2005). There is no 'Savagnin Blanc' growing in this winegrowing region. Plant material from one 'Savagnin Blanc' vine erroneously identified as 'Albariño', sent from Galicia to the National Collection of Grapevine Varieties «El Encín», could be the origin of this confusion. The area planted to 'Albariño' in different Origin Denomination areas is in getting larger and increased from 237 to 2,643 ha between 1987 and 2003 (Consejo Regulador de la Denominación de Origen Rías Baixas, 2004). These results stress the importance of clarifying any mistakes which have been made about this variety. Over the last few years, the commercial importance of 'Albariño', the quality of its wines and the international interest in this variety has been noticeable. Foreign viticulturalists in the USA, Australia and New Zealand, are interested in new plantings of this variety in their home countries.

These results show that 'Albariño' is not the same as 'Savagnin Blanc' or 'Caíño Blanco'. The three grape cultivars are ampelographically clearly different and the current confusion among synonyms came about through similarities shown by shoots and leaves at certain grown stages. The profiles obtained by analysing six microsatellite loci, commonly used in grapevine characterisation, confirmed these results. However, 'Albariño' and 'Caíño Blanco' may be genetically close because they coincided fully at one locus and in one other allele at each of the other loci analysed. Since 'Caíño' is known to be a native of the north-western Iberian Peninsula, 'Albariño' probably shares the same geographic origin.

Table 5. Short and long allele sizes (in base pairs) at each of the six loci analysed

Cultivar	VVMD5	VVMD7	VVMD27	VVS2	ssrVZAG62	ssrVZAG79
Albariño	218 228	237 237	185 185	132 150	185 203	245 249
Savagnin Blanc	228 234	241 255	185 185	150 150	187 193	243 249
Caíño Blanco	218 222	237 261	177 185	140 150	195 203	245 249

The original identification mistake has been corrected at El Encín and recent work on plant material from this collection has included authentic 'Albariño' material (Ibáñez *et al*., 2003; Martín *et al*., 2003, 2006).

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