CHARACTERIZATION OF THE NEARLY EXTINCT 'ALBILLA' CULTIVAR FROM GALICIA AND ITS RELATIONSHIPS WITH OTHER SPANISH 'ALBILLOS'

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Abstract

Aims : This work contributes to the knowledge of the genetic diversity of *Vitis vinifera* L. with the characterization of the non-referenced cultivar 'Albilla' by ampelographic description and molecular markers. This will be useful for the preservation, identification and propagation of this cultivar in the future.

Methods and results : Six microsatellites (SSRs), 55 OIV (International Organisation of Vine and Wine) morphological descriptors and 45 phylometric traits were evaluated in two accessions of 'Albilla' preserved in the Germplasm Bank of EVEGA (Estación de Viticultura e Enoloxía de Galicia), Xunta de Galicia. Additionally, we set out to determine whether this cultivar was distinct from the yet uncharacterized 'Albillo' cultivars found in the Canary Islands. These methods allowed us to describe the cultivar known as 'Albilla' and to compare it with other homonym cultivars.

Conclusions : The 'Albilla' cultivar is different from other genotypes denominated 'Albillo' and any other genotype described until now. This may indicate that it could be an autochthonous cultivar from Galicia.

Significance and impact of the study : The characterization of cultivars is still necessary in order to identify any homonyms or synonyms, develop measures for their conservation (germplasm collection) and evaluate their potential for producing quality wines.

Key words : *Vitis vinifera*, ampelography, microsatellites

Résumé

Objectifs : Ce travail contribue à la connaissance de la diversité génétique de *Vitis vinifera* L. à travers l'évaluation de la variété inconnue 'Albilla' en utilisant une description ampélographique et des marqueurs moléculaires. Cette étude pourra être utile pour la préservation, identification et propagation de cette variété dans le futur.

Méthodes et résultats : Six microsatellites (SSRs), 55 descripteurs morphologiques de l'OIV (Organisation Internationale de la Vigne et du Vin) et 45 caractères phyllométriques ont été évalués sur deux accessions d''Albilla' préservées dans la banque de matériel phytogénétique de l'EVEGA (Estación de Viticultura e Enoloxía de Galicia), Xunta de Galicia. Nous avons de plus déterminé si cette variété était distincte de la variété encore non caractérisée 'Albillo' présente aux Îles Canaries. Ces méthodes nous ont permis de décrire la variété dénommée comme 'Albilla' et de la comparer avec d'autres variétés homonymes.

Conclusions : La variété 'Albilla' est différente des génotypes nommés "Albillo" et distincte des génotypes décrits jusqu'à aujourd'hui. Ceci indique qu'elle pourrait être une variété autochtone de Galicia.

Signification et impact de l'étude : La caractérisation de variétés reste nécessaire pour trouver des homonymes ou synonymes, agir pour leur sauvegarde et évaluer leur potentiel pour produire des vins de qualité.

Mots clés : *Vitis vinifera*, ampélographie, microsatellites

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INTRODUCTION

Geographical diversity, climate conditions and history have contributed to the large number of cultivars that were conserved and spread throughout Europe before the nineteenth century (This *et al.*, 2006), that is, when phylloxera, downy mildew and powdery mildew caused severe genetic erosion.

Galician grapevines originate from western Europe and are composed of a mixture of autochthonous and allochthonous cultivars, mainly of French origin, introduced into the region with the establishment of religious institutions (Huetz de Lemps, 1967). Due to the problems caused by phylloxera and downy and powdery mildew in the nineteenth century, only a few of the most productive cultivars were promoted for wine production, and this process continues. However, autochthonous cultivars are also able to produce both quality and distinct wines. Thus, the identification and characterization of such cultivars is still necessary in order to discover any homonyms or synonyms and evaluate their potential for producing quality wines.

Some traditional cultivars from north-western Spain have recently been characterized, but this analysis did not include 'Albilla' (Santiago *et al.*, 2005). 'Albilla' is a minor cultivar found in north-western Spain. It is in danger of extinction since its potential to produce quality wine is unknown. 'Albilla' has been cultivated since ancient times in the Ribeiro region (Galicia) and has been mentioned by the oldest growers, though the earliest written evidence was found in 'Catastro Vitícola' (1983). 'Albilla' is authorized in the Designation of Origin (D.O.) Ribeiro, but it is not yet included in the National Register of Commercial Cultivars (Cabello *et al.*, 2011). Therefore, it cannot be commercially propagated. 'Albillo' (not 'Albilla') has been reported by García de los Salmones (1914) in eastern Galicia, whereas 'Albilla' has been reported in the 'Catastro Vitícola y Vinícola (D.O. Ribeiro)' (1983) specifically in the localities of Beade and Cenlle, where a few scarce accessions can still be found.

The complexity of the 'Albillo' denomination in Spain goes back over two centuries due to the general meaning of the word (i.e., white). To our knowledge, in Galicia, 'Albillo' was first cited in 1914 by García de los Salmones in eastern Galicia, and more specifically in Fonsagrada (province of Lugo) and Puebla de Trives (province of Ourense). Recently, many authors have studied the complexity of 'Albillo', including synonymies and homonymies. The different genotypes named 'Albillo' cultivated in the Iberian Peninsula, which have been characterized by ampelography and molecular markers (Cervera *et al.*, 2001), are preserved in the grapevine collection of El Encín (Spain): i) 'Albillo' (official prime name 'Albillo Real'), cultivated in Ávila, Madrid, León and Zamora, with its synonyms 'Nieves Temprano' in Zaragoza, 'Temprano de Campo Real' or 'Temprano de Mora' in Madrid, 'Albillo de Toro' in Zamora and 'Albillo de Cebreros' in Ávila and its homonym 'Albillo de Granada' (prime name); ii) 'Albillo Mayor' from Palencia, with its synonyms 'Turruntés' in La Rioja (Rodríguez-Torres *et al.*, 2000), 'Albillo' in Burgos and Valladolid, and 'Blanco del País' in Burgos; and iii) 'Albillo de El Bierzo' (official prime name 'Chasselas' in the Registro de Variedades Comerciales), with its synonyms 'Albillo' in Asturias and Salamanca, 'Malvasía' in León, and 'Temprano' or 'Temprano Blanco' in Valladolid.

Cabello *et al.* (2003) have reported that the 'Albillo' cultivated in Galicia is a synonym of 'Chasselas Doré'. Rodríguez-Torres *et al.* (2000) considered the 'Albillo' cultivated in the D.O. Ribera del Duero to be synonymous with the 'Turruntés' from the D.O. La Rioja. Martínez and Pérez (2000) have described 'Albillo' in Asturias, which has been recently identified as 'Chasselas'(Moreno-Sanz *et al.*, 2008).

The basic methodology for cultivar characterization is ampelography complemented with the analysis of molecular markers, mainly microsatellites (SSRs) (Thomas *et al.*, 1994). The International Organisation of Vine and Wine (OIV) provides a list of morphological descriptors and recommends the analysis of six microsatellite loci for cultivar identification (OIV, 2009).

In this context, the aim of this study was to characterize the 'Albilla' cultivar conserved in the Germplasm Bank of EVEGA, Xunta de Galicia. Ampelography and microsatellite analysis were used in order to establish the distinctiveness and ampelographical characteristics of the cultivar so that it could be registered in the future and to contribute towards maintaining the genetic diversity of the area. Additionally, we determined whether this cultivar has been present in the Canary Islands, considered as a refuge for Spanish cultivars, where phylloxera would not have affected it (Zerolo *et al.*, 2006).

MATERIALS AND METHODS

1. Plant material

The plant material used in this study comprises two accessions of 'Albilla' from EVEGA, located in Leiro-Ourense (Galicia, Spain) and three accessions named 'Albillo Criollo', 'Albillo Forastero' and 'Albillo

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Monte Lentiscal' from the collection of ICIA (Tenerife, Canary Islands, Spain). The 'Albilla' accessions at EVEGA were obtained by clonal propagation of one-hundred-year-old grapevines from the D.O. Ribeiro (north-western Spain). Two French cultivars from the EVEGA collection were included as references for the SSR analyses : 'Pinot noir' and 'Syrah'.

2. Morphological analysis

Different traits, corresponding to young shoots, mature leaves, clusters and berries, were studied in 2006, 2007 and 2009 on the two accessions of 'Albilla', according to the descriptors defined by the OIV (2009).

Ten leaves from the middle third of the shoot were collected from each accession and evaluated for 45 phylometric traits, as proposed by Cid-Alvarez *et al.* (1994) using NIS-Elements BR 2.30 software.

For the quantitative analysis of leaf parameters, we estimated the average, the coefficient of variation and the significant differences by ANOVA between the accessions studied, using SPSS 15.1 software (SPSS Inc for Windows, Chicago, IL, USA).

3. Molecular analysis

The six SSRs recommended by the OIV (2009) were used in this study. Polymerase chain reaction (PCR) was conducted according to the methodology defined by Martín *et al.* (2003). The PCR products were separated and analysed on a 3730 DNA Analyser (Applied Biosystems, USA). The size of the amplified products was determined using the Peak Software version 1.0 (http://www.appliedbiosystems.com).

RESULTS AND DISCUSSION

Both 'Albilla' accessions from EVEGA showed the same genotype based on six SSRs and were different from 'Albillo Criollo', 'Albillo Forastero', and 'Albillo Monte Lentiscal' from ICIA (Table 1). They were also different from 'Chasselas' from EVEGA, which corresponded to the genotype from the 'Colección de El Encín' reported by Martín *et al.* (2003). Therefore, 'Albilla' from EVEGA is different from the other cultivars previously reported (http://www.sivvem.monbyte.com/sivvem.asp ; http://www.vivc.de ; Rodríguez-Torres *et al.*, 2000 ; Borrego *et al.*, 2002 ; Cabello *et al.*, 2003 ; Ibañez *et al.*, 2003 ; Martín *et al.*, 2003 ; Jiménez-Cantizano *et al.*, 2006 ; Santana *et al.*, 2008 ; Díaz-Losada *et al.*, 2012).

Table 1. Allele sizes (bp) of six microsatellites in two accessions of 'Albilla' from EVEGA (Galicia, in bold), three 'Albillo' cultivars from ICIA (Tenerife) and three French references found in EVEGA.

Cultivar name	Origin	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VrZAG47	VrZAG47	VrZAG62	VrZAG62	VrZAG79	VrZAG79
'Albilla'	**EVEGA Collection**	**130**	**150**	**234**	**236**	**237**	**241**	**165**	**169**	**187**	**195**	**237**	**243**
'Albilla'	**EVEGA Collection**	**130**	**150**	**234**	**236**	**237**	**241**	**165**	**169**	**187**	**195**	**237**	**243**
'Albillo Criollo'	ICIA Collection	130	130	218	236	237	237	161	165	187	195	249	249
'Albillo Forastero'	ICIA Collection	130	142	218	224	237	255	165	171	187	193	249	255
'Albillo Monte Lentiscal'	ICIA Collection	142	150	232	236	237	245	157	159	185	187	245	245
'Pinot noir'	EVEGA Collection	134	150	224	234	237	241	161	165	187	193	237	243
'Syrah'	EVEGA Collection	130	130	222	228	237	237	165	167	187	193	243	249
'Chasselas'	EVEGA Collection	130	140	224	232	237	245	161	165	193	203	249	257

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The ampelographic characteristics of shoots, young and mature leaves, bunches and berries of 'Albilla' are presented in Table 2 and Figure 1. Ampelography confirmed the singularity of 'Albilla' observed by microsatellites with respect to other 'Albillo' types reported in Spain. Cabello *et al.* (2003) identified 'Albillo' as a synonym of 'Chasselas'; however, 'Chasselas' presents bronze young leaves with very low density of hairs and mature leaves with very deep lateral sinuses, as opposed to 'Albillo' from EVEGA. 'Albillo Mayor', cultivated in the D.O. Ribera del Duero and Cigales (Rubio *et al.*, 2003), can be distinguished from the 'Albillo' maintained at EVEGA by its large mature leaves with 7 lobes, very overlapped petiole sinus and lateral sinuses, cylindrical bunches, and berries with Muscat flavor. Finally, 'Albillo' from EVEGA is different from 'Albillo Real' (Rubio *et al.*, 2003; Cabello *et al.*, 2003) found in Madrid, Zamora and Ávila since the latter presents overlapped lateral sinuses, opened petiole sinus, and spherical berries.

Table 3 gives the phylometric measurements of 'Albilla' leaves. Both of the 'Albilla' accessions from EVEGA presented 77.7% of the ampelographic traits with no significant differences, which indicates the stability of these traits. The main significant variations were found in: the lengths of the central vein and the upper and lower lateral main veins (N1-OIV 601, N2-OIV 602, N3-OIV 603); the number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein (ND-OIV 616); the distance between the petiole point and upper sinus (U-OIV 605); the length of the tooth N1 to the half point at its base (L1); the distance between the tips of N2 and N3 (ENM); the projection of the distance between N3 and N1 over the leaf axis (L); the sum of the lengths N2 on the large side and N1 on the small side of the leaf (N2S); the angle between the extremes of N1 and N2 (AP); and the distance between the bases of the upper sinuses (DS1). These variations can be considered as clonal variations.

Microsatellite genotyping and ampelographic data showed that 'Albilla' from EVEGA, propagated from Galician grapevines that are more than 100 years old, is different from the grapevine preserved in the 'Colección de Variedades de Vid de El Encín' and is also different from 'Albillo Criollo', 'Albillo Forastero' and 'Albillo Monte Lentiscal' from the Canary Islands. Therefore, 'Albilla' from EVEGA is a minor cultivar. 'Albillo' is the name cited in previous references and corresponds to the genotypes included in the European Vitis Database, but we have used the name 'Albilla' because this is the name given by the growers to the genotype from EVEGA, the true-to-type variety authorized in the D.O. Ribeiro, which is different from other genotypes named 'Albillo'.

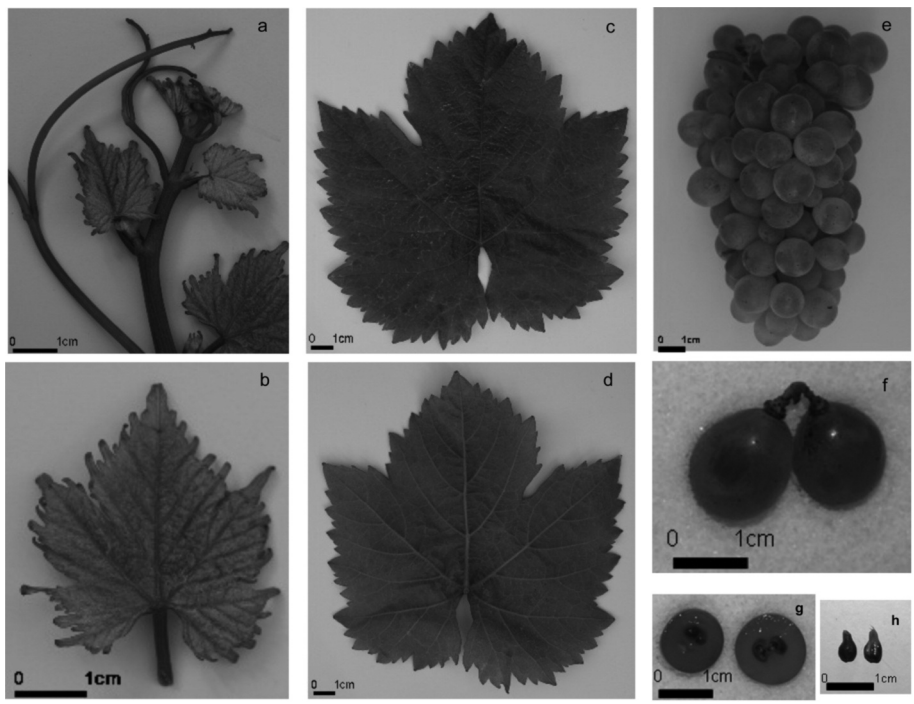


Figure 1 -Young shoot (a), upper side of young leaf (b), upper side of mature leaf (c), lower side of mature leaf (d), cluster (e), berries (f), cross-section of the grape berry (g) and seeds (h) of 'Albilla' from EVEGA, Galicia.

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Table 2 - Ampelographic traits analysed in a young shoot, mature leaf, bunch and berry of the 'Albilla' cultivar (OIV, 2009) (Expression indicates mode with minimum and maximum values in parenthesis).

No. Code	Characteristics	Expression	Notes
001	Young shoot: aperture of tip	3	fully open
002	Young shoot: distribution of anthocyanin coloration on prostrate hairs of tip	2	piping
003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of tip	5 (5-7)	medium
004	Young shoot: density of prostrate hairs on tip	3	low
005	Young shoot: density of erect hairs on tip	1	none or very low
006	Shoot: attitude (before tying)	3	semi-erect
007	Shoot: color of dorsal side of internodes	2	green and red
008	Shoot: color of ventral side of internodes	1	green
009	Shoot: color of dorsal side of nodes	2	green and red
010	Shoot: color of ventral side of nodes	1 (1-2)	green
011	Shoot: density of erect hairs on nodes	1	none or very low
012	Shoot: density of erect hairs on internodes	1	none or very low
015.2	Shoot: intensity of anthocyanin coloration on bud scales	1	none or very low
051	Young leaf: color of the upper side of blade (4th leaf)	3	bronze
053	Young leaf: density of prostrate hairs between main veins on lower side of blade	5 (5-3)	medium
056	Young leaf: density of erect hairs between main veins on lower side of blade	1	none or very low
065	Mature leaf: size of blade	5 (5-7)	medium
067	Mature leaf: shape of blade	3 (2-3)	pentagonal
068	Mature leaf: number of lobes	3	five
069	Mature leaf: color of the upper side of blade	5	medium
070	Mature leaf: area if anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcat
071	Mature leaf: area if anthocyanin coloration of main veins on lower side of blade	3	up to the 1st bifurcation
072	Mature leaf: goffering of blade	3 (3-1)	weak
073	Mature leaf: undulation of blade between main and lateral veins	1 (1-9)	absent
074	Mature leaf: profile of blade in cross section	5	twisted
075	Mature leaf: blistering of upper side of blade	3	weak
076	Mature leaf: shape of teeth	2 (2-5)	both sides straight
078	Mature leaf: length of teeth compared with their width	5 (3-5)	medium
079	Mature leaf: degree of opening / overlapping of petiole sinus	4 (3-4)	slightly open
080	Mature leaf: shape of base of petiole sinus	3	V-shape
081.1	Mature leaf: teeth in the petiole sinus	1	none
081.2	Mature leaf: petiole sinus limited by vein	1	not delimited
082	Mature leaf: degree of opening / overlapping of upper lateral sinuses	1	open
083.1	Mature leaf: shape of base of upper lateral sinuses	3	V-shape
083.2	Mature leaf: teeth in the upper lateral sinuses	1	none
084	Mature leaf: density of prostrate hairs between the main veins on lower side of blade	3(3-4)	low
085	Mature leaf: density of erect hairs between the main veins on lower side of blade	1	none or very low
086	Mature leaf: density of prostrate hairs on main veins on lower side of blade	1 (1-3)	none or very low
087	Mature leaf: density of erect hairs on main veins on lower side of blade	3 (1-4)	low
088	Mature leaf: prostrate hairs on main veins on upper side of blade	1	absent
089	Mature leaf: erect hairs on main veins on upper side of blade	1	absent
090	Mature leaf: density of prostrate hairs on petiole	1	none or very low
091	Mature leaf: density of erect hairs on petiole	1	none or very low
093	Mature leaf: length of petiole compared to length of middle vein	3	slightly shorter
202	Bunch: length (peduncle excluded)	5	medium
203	Bunch: width	5	medium
204	Bunch: density	5	medium
206	Bunch: length of peduncle of primary bunch	3 (3-5)	short
208	Bunch: shape	2	conical
209	Bunch: number of wings of the primary bunch	2	1-2 wings
220	Berry: length	3	short
221	Berry: width	3	narrow
222	Berry: uniformity of size	2	uniform
223	Berry: shape	3	short elliptic
225	Berry: color skin	1	green yellow

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Table 3 - Leaf traits of the 'Albilla' cultivar from EVEGA (mean and F values, standard deviation [S.D.], coefficient of variation [C.V.] in %). The references from OIV are shown in parentheses.

Code	Characteristics	Mea	S.D	C.V.	F	Significan
N1 (OIV	Length of central vein	12.45	0.9	7.64	7.77	***
N2 (OIV	Length of upper lateral main vein	10.86	0.6	5.53	3.91	**
N3 (OIV	Length of lower lateral main vein	7.67	0.4	6.22	3.46	*
N4 (OIV	Length of lower secondary vein	5.18	0.2	4.30	1.68	n.s.
N5 (OIV	Length of lower tertiary vein	2.51	0.0	2.56	0.09	n.s.
N6	Distance between petiole point and intersection N3-N4	0.83	0.0	7.63	0.56	n.s.
U (OIV 605)	Distance between petiole point and upper sinus	8.59	0.9	11.4	15.5	***
O (OIV 606)	Distance between petiole point and lower sinus	6.65	0.6	9.99	1.02	n.s.
ND (OIV	Number of teeth between the tooth tip of N2 and the tooth tip of the first	8.66	0.7	8.89	9.09	***
PL	Length between the tooth tip of N2 and the tooth tip of the first secondary	5.50	0.4	8.67	3.04	n.s.
L1	Length of tooth N1 to the half point at its base	0.60	0.1	17.4	6.81	***
L2 (OIV 612)	Length of tooth N2 to the half point at its base	1.06	0.3	28.4	0.74	n.s.
L3	Length of tooth N3 to the half point at its base	0.79	0.0	9.01	2.21	n.s.
L4 (OIV 614)	Length of tooth N4 to the half point at its base	0.84	0.0	9.89	2.18	n.s.
A1	Width of tooth N1	1.01	0.0	6.27	0.40	n.s.
A2 (OIV	Width of tooth N2	1.25	0.1	8.82	1.90	n.s.
A3	Width of tooth N3	1.69	0.8	48.6	1.21	n.s.
A4 (OIV	Width of tooth N4	1.34	0.3	29.2	1.04	n.s.
SP (OIV 618)	Degree of opening of petiole sinus	1.37	0.0	4.44	0.00	n.s.
AN	Width of the leaf	16.26	0.3	1.87	0.46	n.s.
ALT	Length of the leaf	16.14	0.1	0.63	0.00	n.s.
L	Projection of the distance between N3 and N1 over the leaf axis	14.71	1.2	8.46	6.08	**
IS	Distance between the tips of N2g-N2p	14.09	0.7	4.96	0.36	n.s.
II	Distance between the tips of N3g-N3p	15.04	0.7	4.70	0.50	n.s.
ENS (OIV	Distance between the tips of N1-N2	8.44	0.3	3.94	0.88	n.s.
ENM	Distance between the tips of N2-N3	8.51	0.6	7.93	3.41	*
ENI	Distance between the tips of N3-N4	5.99	0.4	7.76	2.81	n.s.
NL	Distance between petiole point and tip N4	5.88	0.2	3.90	1.90	n.s.
N2S	Sum of the lengths N2g-N2p	21.45	1.6	7.61	4.50	**
DS1	Distance between the bases of the upper sinuses	8.36	0.7	8.72	4.75	**
DS2	Distance between the bases of the lower sinuses	13.46	1.1	8.39	0.98	n.s.
DS	Distance between the bases of the lower and the upper sinuses	7.86	1.5	19.1	2.97	n.s.
SS	Degree of opening of upper sinus	0.49	0.0	10.2	0.14	n.s.
SI	Degree of opening of lower sinus	1.14	0.6	56.2	2.72	n.s.
A (OIV 607)	Angle between N1 and N2	50.61	0.6	1.27	0.13	n.s.
B (OIV 608)	Angle between N2 and N3	53.13	3.1	5.87	2.55	n.s.
C (OIV 609)	Angle between N3 and N4	53.12	0.5	1.03	0.07	n.s.
D (OIV 610)	Angle between N4 and N5	43.96	1.1	2.70	0.30	n.s.
E	Angle between N3 and the edge of petiole sinus	65.41	4.6	7.15	0.78	n.s.
F	Angle between the extremes of N1 and its first secondary vein	101.5	4.6	4.58	2.08	n.s.
AP	Angle between the extremes of N1 and N2	119.3	5.7	4.81	7.53	***
AB	A+B	103.7	2.4	2.39	1.84	n.s.
ABC	A+B+C	156.8	2.0	1.31	0.45	n.s.
ABE	A+B+E	169.1	6.5	3.86	1.85	n.s.
CD	C+D	97.08	1.6	1.67	0.27	n.s.

n.s., *, **, *** non-significant and significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

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García de los Salmones (1914) cited 'Albillo' in eastern Galicia, while the 'Albilla' from EVEGA was localised in the Ribeiro region. There is no description for the 'Albillo' cited by García de los Salmones (1914) to compare with the 'Albilla' from EVEGA. The 'Albillo' genotypes from previous studies are different from the 'Albilla' genotype included in the EVEGA and used in the Ribeiro region, where it is still cultivated and cited by the oldest growers. Moreover, in the 'Catastro Vitícola' (1983), it is included with the denomination 'Albilla' from Ribeiro. For all these reasons, we recommend maintaining the name 'Albilla of Ribeiro' (the name of the accessions in the EVEGA collection) to avoid any confusion with other genotypes named 'Albillo'.

CONCLUSIONS

This study provides descriptive data of the 'Albilla' cultivar, which is currently in danger of extinction due to the impossibility of commercializing unregistered plants for new plantations, even though it has been considered a preferred variety in the D.O. Ribeiro. This cultivar is different from other genotypes denominated 'Albillo' and any other genotype described until now, as proved by the six SSR analysis. This may indicate that it could be an autochthonous cultivar from Galicia.

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