UNKNOWN GRAPEVINE GENETIC RESOURCES IN ASTURIAS (SPAIN) ON THE EDGE OF EXTINCTION

Paula MORENO-SANZ[1], María Dolores LOUREIRO[2*] and Belén SUÁREZ[2]

1 : Research and Innovation Center - Fondazione Edmund Mach, Department of Genomics and Biology of Fruit Crops - Grapevine Applied Genomics, Via E. Mach, 1 - 38010 San Michele all'Adige (TN), Italy
2 : Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Área de Tecnología de los Alimentos, P.O. Box 13 - 33300 Villaviciosa, Asturias, Spain

Abstract

Aim : Grapevine is a traditional crop in Asturias (northern Spain). However, an important part of the varietal heritage of this region is still unknown. Great biodiversity was reported in previous surveys, with varietal identification being carried out via microsatellite markers. The aim of the present study was to confirm the previous genetic identification and provide the first description of the unknown cultivars.

Methods and results : Forty cultivars were described *in situ* over a two-year period through 58 ampelographic descriptors. A model description was performed for each cultivar and great variability was found : white, red and rosé grape cultivars ; hybrids ; winemaking and dual-use (wine and table grapes) cultivars ; and some teinturier cultivars. Cluster analysis grouped cultivars with similar characteristics.

Conclusion : Ampelographic description was complementary to molecular identification in confirming the identity of the studied cultivars. Moreover, unknown cultivars have been described for the first time. Complete characterization is necessary because these cultivars constitute valuable genetic resources for crop breeding programmes.

Significance and impact of the study : Considering the limited number of accessions of the unknown cultivars and the replanting of old vineyards with vines approved by *Cangas Quality Wine* regulations, their conservation and study is vital to provide genetic resources for potential breeding or technological purposes in the future.

Key words : grapevine, minor cultivar, ampelography, Asturias

Résumé

Objectif : La vigne des Asturies est une culture traditionnelle dans cette région du nord de l'Espagne, dont une partie importante du patrimoine variétal reste inconnue à ce jour. Des études scientifiques y ont observé une grande biodiversité, dont l'identification variétale a été établie avec des marqueurs microsatellites. Le but du présent travail est de confirmer ces identifications génétiques et d'offrir une première description des cultivars inconnus.

Méthodes et résultats : Quarante cultivars ont été décrits *in situ* sur une période de deux ans à travers 58 descripteurs ampélographiques, ce qui a permis de réaliser une description standard pour chaque cultivar. Lors de ce travail de description, il a été constaté une grande variabilité ampélographique : ont été observés, en effet, des cultivars à grains blancs, rouges et rosés ; des cultivars hybrides ; des cultivars spécialement indiqués pour l'élaboration du vin et d'autres pouvant se destiner à un double usage (raisins de cuve et raisins de table) ; enfin, certains cultivars teinturiers. Une analyse typologique a permis de regrouper les variétés qui présentaient des caractéristiques similaires.

Conclusion : La description ampélographique et l'identification moléculaire sont deux procédés complémentaires qui ont permis de confirmer l'identité génétique des cultivars étudiés. Des cultivars inconnus ont par ailleurs été décrits pour la première fois. Leur caractérisation complète est nécessaire dans la mesure où ils constituent une ressource génétique à considérer dans des programmes d'amélioration génétique.

Signification et impact de l'étude : Compte tenu du nombre limité de plants trouvés pour chacun des cultivars non identifiés et la replantation de vignobles anciens avec des vignes approuvées par le label *Vin de Qualité de Cangas*, la conservation et l'étude sont primordiales pour sauvegarder les ressources génétiques et contribuer à la poursuite de recherches technologiques.

Mots clés : vigne, variété mineure, ampélographie, Asturies

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*Corresponding author : mdolorlr@serida.org

INTRODUCTION

The OIV/VITI 424/2010 resolution recommends to the member countries the surveillance, identification and conservation of grapevine material that has not yet been described or characterized, because of the risk of genetic erosion and extinction of such cultivars. In this regard, all the countries with a longstanding tradition of growing grapes have undertaken these tasks in recent years with the aim of detecting minor cultivars, well appreciated nowadays on account of their potential to provide a greater diversification of wines (González-Andrés *et al.*, 2007; Gago *et al.*, 2009; Sabir *et al.*, 2009; Ates *et al.*, 2011; García-Muñoz *et al.*, 2012). For example, Spanish minor cultivars such as Albariño, Prieto Picudo and Godello have been recovered and are being increasingly used to produce quality wines.

Vitis vinifera L. has suffered considerable genetic erosion since the late 19th century; first, because of the phylloxera plague, and later due to the homogenization of the wine market, which has led to the replanting of vineyards with well-known cultivars and hence the loss of many autochthonous ones. Although there are around 10,000 cultivars in germplasm banks, the actual number of different cultivars worldwide is estimated to be around 5,000, a difference due to the existence of synonyms and homonyms in preserved plant material (This *et al.*,

Table 1 - Cultivars quoted in bibliographic references.

Berry colour	Suárez (1879)	García (1914)	Naredo (1914)
Blue / black	Agudiello=Negrón *Alvarín negro*=Pata de Perdiz Carrasquín Verdejo Carrasco	Agudillo *Alvarín* Carrasquín Verdejo Conrasión Negrín Negrón Rondales Mallén Pardusco Prieto Pata de Perdiz Picudo	Agudiello *Alvarín Negro* Carrasquín Verdejo Carrascón Negrín Negrón Rondal Negro Alicante Cabernet Garnacha Tintorera Malbec Mencía Sumoll
Green / yellow	*Alvarín Blanco*=Albillo Moscatel Teta de vaca	Albarín Moscatel Bondal Pedro Jiménez Verdeja	*Alvarín Blanco* Moscatel
Rose / red			Jaén – Moscatel Garnacha Roja

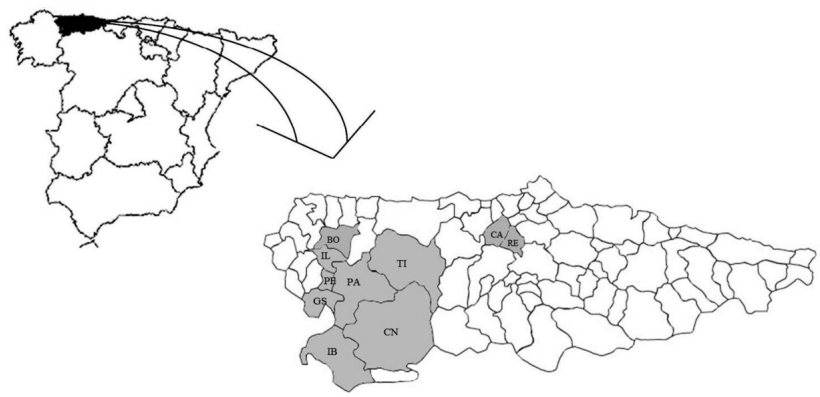

Figure 1 - Boroughs surveyed in Asturias (Spain). BO, Boal; IL, Illano; PE, Pesoz; GS, Grandas de Salime; PA, Pola de Allande; IB, Ibias; CN, Cangas del Narcea; TI, Tineo; CA, Candamo; RE, Las Regueras.

2006). Despite this broad diversity, only a small number of these cultivars occupy a large proportion of vineyard lands.

Asturias is located in northern Spain. The first evidence of grapevine cultivation in this region dates back to 781 (Cortizo *et al.*, 2008). In 1858, vineyards occupied an area of 5,493 ha (Feo, 1986), but the phylloxera plague, cryptogamic diseases, and later the expansion of coal-mining activities led to a significant reduction in the cultivated area, which now covers about 123 ha. Moreover, old vineyards are difficult to manage due to the rugged terrain and the steep slopes. This fact, together with the aging of the region's vine growers and the recognition in 2008 of the region-specific wine appellation *Cangas Quality Wine*, is leading to the abandonment of old vineyards or their replanting with cultivars approved by the wine appellation regulations, with the consequent reduction in diversity.

Cultivars cited in old references are listed in Table 1. More recently, in 2005, the Regional Agrofood Research and Development Service of Asturias (« *Servicio Regional de Investigación y Desarrollo Agroalimentario of Asturias - SERIDA* ») launched a survey of cultivar diversity across all Asturian boroughs with an old viticulture tradition. The accessions found were analysed via microsatellite markers (SSRs) and the genetic profiles thus obtained were compared with national and international databases. As a result, 40 different cultivars were found, of which only 28 could be identified (Moreno-Sanz *et al.*, 2011). Four cultivars were later discovered and analysed using microsatellite markers.

MATERIALS AND METHODS

1. Plant material

Surveys were carried out in vineyards more than 50 years old located in the southwest of Asturias (Figure 1), in two phases (2005-2007 and 2009-2010). Plants were selected covering all the observed phenotypic variability and taking into account the different names given to the accessions by the vine growers (whenever possible). Surveys were also carried out in inner boroughs where cultivated vineyards existed in the past, although only some isolated plants remain today.

2. Microsatellite analysis

The plants selected in the 2005-2007 surveys were genotyped in previous studies. The accessions surveyed in 2009 and 2010 were analysed using microsatellite loci. DNA extraction and PCR protocol were carried out in accordance with Moreno-Sanz *et al.* (2011). The following SSR markers were used: VVS2 (Thomas and Scott, 1993), VVMD7 (Bowers *et al.*, 1996), VVMD27, VVMD28 (Bowers *et al.*, 1999b), VrZAG62, VrZAG67, VrZAG79 and VrZAG112 (Sefc *et al.*, 1999). SSR profiles were compared with the national and international databases listed in Moreno-Sanz *et al.* (2011).

3. Ampelographic description

Ampelographic descriptions were carried out *in situ* for two consecutive years (2008-2009 and 2010-2011) by two ampelographers, following the methodology of the « *OIV descriptor list for grape varieties and Vitis species* » (OIV, 2008). Ten accessions of each cultivar were described. In the cases in which less than ten accessions were surveyed, ten organs were described in all for each parameter. In total, 58 ampelographic descriptors were recorded (Table 2).

A model description was developed for each cultivar by selecting the mode within the values obtained for each descriptor.

4. Statistical analysis

A data matrix was constructed with the model description of each variety. A hierarchical cluster analysis was performed using NTSYS v.2.2 software (Rohlf, 2005). The dendrogram was obtained using the UPGMA method, applying the Jaccard coefficient.

Table 2. Ampelographic descriptors studied.

Part of plant	OIV code		
Young Shoot	OIV 001		
	OIV 002		
	OIV 003		
	OIV 004		
Shoot	OIV 007	OIV 011	OIV 015-1
	OIV 008	OIV 012	OIV 015-2
	OIV 009	OIV 013	OIV 016
	OIV 010	OIV 014	OIV 017
Young leaf	OIV 051		
	OIV 053		
	OIV 054		
Mature leaf	OIV 067	OIV 079	OIV 085
	OIV 068	OIV 080	OIV 086
	OIV 069	OIV 081-1	OIV 087
	OIV 070	OIV 081-2	OIV 090
	OIV 072	OIV 082	OIV 091
	OIV 074	OIV 083-1	OIV 094
	OIV 075	OIV 083-2	
	OIV 076	OIV 084	
Bunch	OIV 202	OIV 207	
	OIV 203	OIV 208	
	OIV 204	OIV 209	
	OIV 206		
Berry	OIV 220	OIV 225	OIV 236
	OIV 221	OIV 226	OIV 238
	OIV 222	OIV 227	
	OIV 223	OIV 231	

Table 3. Genetic profiles (allele sizes in bp) of the cultivars discovered in the surveys carried out in 2009 and 2010.

Cultivar	SSR markers															
	VVS2		VVMD7		VVMD27		VVMD28		VrZAG62		VrZAG67		VrZAG79		VrZAG112	
Cabernet Sauvignon*	135	148	240	240	173	186	234	236	187	193	126	140	248	248	232	236
Mazuelo	139	141	240	240	178	182	248	258	185	187	126	140	252	259	232	240
Moscatel de Alejandría	129	146	250	252	176	191	244	267	185	203	126	126	248	256	236	250
GEN 19	129	146	244	248	168	176	234	244	191	203	140	156	255	257	232	250
GEN 20	129	148	240	256	176	186	236	246	185	193	132	132	246	252	242	242

* Reference cultivar; GEN, unidentified cultivar

The cophenetic correlation coefficient was also calculated.

RESULTS

The genetic profiles of the four cultivars discovered in 2009 and 2010 and their identification according to the searched databases are listed in Table 3. Two corresponded to Mazuelo and Moscatel de Alejandría, one (GEN 19) could be the Italia cv. (it shows an allelic variation for the VVMD27 marker), and one (GEN 20) remains unidentified. All the cultivars that could not be identified are coded as GEN and a number.

These four cultivars, together with 36 of the cultivars discovered between 2005 and 2007 and already genotyped in a previous study (Moreno-Sanz *et al.*, 2011) were ampelographically described (Table 4).

The model description of each cultivar is shown in Tables 5 to 7. Descriptors with no variability (i. e., the notation was 1 for all the accessions) were eliminated from the analysis (OIV 011, OIV 012, OIV 016). Descriptors OIV 017, OIV 204 and OIV 222 for the cultivars GEN 20 and Mazuelo, OIV 204 and OIV 222 for GEN 19, OIV 236 for Roseti, and all the descriptors of bunch and berry for Italia were not included in the analysis.

The dendrogram obtained from the data matrix is shown in Figure 2. The cophenetic correlation coefficient was 0.592. Seven groups appear at a similarity level of 0.38. Groups I and V have only two cultivars each. Group II comprises supposed autochthonous cultivars from the region (Albarín Tinto, Carrasquín, Verdejo Tinto), as well as Spanish, foreign and five unidentified cultivars. Group III comprises Cardinal, Roseti and Italia, which are dual-use cultivars (wine and table grapes), three unknown cultivars, and Mencía. Group IV also includes dual-use cultivars (Chasselas and Moscatel groups and Aramon cv.), together with De José Blanco and three unidentified cultivars. Group VI associates the teinturier cultivars, together with Palomino, while Cabernet-Sauvignon is very different from the other cultivars and is found in a distinct group (Group VII).

Table 4. Ampelographically described cultivars and their location.

	Cultivar	Boroughs
Red	Albarín Tinto	CN, IB, IL, GS, PE
	Aramon*	CN, PE
	Cabernet Sauvignon	CN
	Cardinal*	IL, BO
	Carrasquín	CN, IB
	Garnacha Tintorera	CN, IB, IL
	Mazuelo*	CN
	Mencía	CN, IB, IL, GS, PE, TI
	Morenillo II*	CN, PE
	Morrastel Bouschet*	IB
	Mouratón	CN, GS, IL, PE, TI
	Petit Bouschet	CN
	Sumoll*	CN
	Verdejo Tinto	CN, IB, GS, PE, RE
	GEN 08*	CN
	GEN 10*	CN
	GEN 12*	IB, GS
	GEN 13*	GS
	GEN 14*	IB
	GEN 19*	CN
White	Albarín Blanco	CN, IB, IL, GS, PE
	Chasselas Doré	CN, IB
	De José Blanco*	IB, IL
	Doña Blanca	CN, IB, PE
	Furmint*	CN
	Godello	CN, IB, IL
	Italia*	IL
	Lairén*	PE
	Moscatel Blanco de grano menudo	CN
	Moscatel de Alejandría*	GS
	Palomino	CN, IB, IL, PE, TI, BO
	Roseti*	IL
	Savagnin Blanc	CN, IB
	GEN 01*	IB, GS, PE
	GEN 05*	CN
	GEN 06*	IB
	GEN 20*	CN
Rosé	Chasselas Rosé*	IB
	Moscatel Rojo	CN
	GEN 16	CN

GEN: unidentified cultivar. BO, Boal; CN, Cangas del Narcea; GS, Grandas de Salime; IB, Ibias; IL, Illano; RE, Las Regueras; PE, Pesoz; TI, Tineo. * Cultivars with less than 10 accessions found

Table 5. Values obtained for each descriptor of young shoot, shoot and young leaf.

Cultivar	Young shoot, shoot and young leaf															
	OIV 001	OIV 002	OIV 003	OIV 004	OIV 007	OIV 008	OIV 009	OIV 010	OIV 013	OIV 014	OIV 15-1	OIV 15-2	OIV 017	OIV 051	OIV 053	OIV 054
Albarín Blanco	5	2	1	5	2	2	2	1	1	1	2	1	3	3	5	1
Albarín Tinto	5	2	3	7	2	1	2	1	3	1	2	7	5	1	9	1
Aramon	5	2	3	5	2	1	2	1-2	1	1	2	5	3	3	5	1-5
Cabernet Sauvignon	5	2	7	5	2	2	1	1	1	1	2	9	3	4	7	1
Cardinal	3-5	2	7	1	2	1-2	2	1	1	1	1	1	9	4	1	1
Carrasquín	5	2	3	9	2	1	1	1	3	3	1	1	7	3	9	1
Chasselas Doré	5	2	9	3	2	1	2	1	1	1	3	7	7	4	3	1
Chasselas Rosé	5	2	3-7	3	2	2	2	2	1	1	3	5	5	4	1	1
De José Blanco	5	2	1	5	2	1	1	1	1	1	1	1	5	1	5	1
Doña Blanca	5	3	7	7	1	1	1	1	3	3	1	1	5	1	7	1
Furmint	5	2	3	7	2	2	2	2	3	5	2	7	1	3	7	1
Garnacha Tintorera	5	2	5	5-7	2	1	2	1	5	3	1	1	3	3	7	1
GEN 01	5	2	3	5	2	1	2	1	1	1	2	5	1	3	5	1
GEN 05	5	2	3	5	2	1-2	2	1-2	1	1	2	5	3	3	3	1
GEN 06	5	3	3	5	3	2	3	2	1	1	3	3	1	3-4	5	1-9
GEN 08	5	2	3	7	2	1	2	2	3	1	1	1	3	3	3	1
GEN 10	5	3	7	5	2	1	2	1	1	1	1	1-7	3	1	7	1
GEN 12	3	2	5	5	2	2	2	2	1	3	1	1	3	1	1	1
GEN 13	3	1	1	1	1	1	1-2	1	1	1	1	1	3	1	1	1
GEN 14	5	3	9	5	3	1-2	1	1	1	1	1	1	9	1	3	1
GEN 16	5	1	1	7	2	1	1	1	3	3	1	1	5	1	9	1-3
GEN 19	5	1	1	3	1	1	1	1	1	1	1	1	5	1	3	1
GEN 20	5	2	5	5	2	1	2	1	1	1	1	1	-	1	5-7	5
Godello	5	3	5	5	2	1	1	1	1	1	1	1	7	1	7	1
Italia	5	2	1-5	7	2	1	2	1	3	1	1	1	9	3	5	1
Lairén	5	2	3	7	2	1	1	1	3	1	1	1	5	3	7	1
Mazuelo	5	1	1	5-7	2	2	1	1	1	1	1	1	-	1	5-7	3
Mencía	5	1	1	3	2	1	1-2	1	1	1	1	1	3	1	1	1
Morenillo II	5	3	7	3	1	1	1	1	3	3	1	1	3	3	5	3
Morrastel Bouschet	5	1	1	7	2	2	2	2	5	7	1-2	1	5	3	7	1
Moscatel Blanco	5	2	3	5	2	1	1	1	3	1	2	7	3	4	3	1
Moscatel Rojo	5	2	3	3	2	1	1	1	1	1	2	7	5	4	3	1
Moscatel de Alejandría	5	1	1	5	1-2	1	1	1	1-3	1	2-3	7	7	3-4	1-3	1
Mouratón	5	2	1	7	1	1	1	1	3	3	1	1	5	1	7	1
Palomino	5	2	3	7	2	1	2	1	5	5	1	1	5	1	7	1
Petit Bouschet	5	2	7	7	2	1-2	2	1-2	1	1	3	7	5	4	7	1
Roseti	5	2	7	1	1	1	1	1	1	1	1	1	7	3	1	1
Savagnin Blanc	5	2	5	5	2	1-2	2	1	1	1	2	1	3	3	5	1
Sumoll	5	2	3-5	3-5	1-2	1-2	1	1	1	1-3	1	1	3	3	5	1
Verdejo Tinto	5	2	5	5-7	2	1	2	1	3	1	2	7	5	3	9	1

Table 6. Values obtained for each descriptor of mature leaf.

	Mature leaf																				
Cultivar	**OIV 067**	**OIV 068**	**OIV 069**	**OIV 070**	**OIV 072**	**OIV 074**	**OIV 075**	**OIV 076**	**OIV 079**	**OIV 080**	**OIV 081-1**	**OIV 081-2**	**OIV 082**	**OIV 083-1**	**OIV 083-2**	**OIV 084**	**OIV 085**	**OIV 086**	**OIV 087**	**OIV 090**	**OIV 091**	**OIV 094**
Albarín Blanco	3	1-2	5	3	1	1	5	2	3	3	1	1	1	3	1	3	3-5	3	1	1	1	1
Albarín Tinto	4	1	7	2	3-5	5	5	5	7	3	1	1	1	3	2	5	3	3	1	1	1	1
Aramon	3	3	5	3	1	5	5	5	3	3	1	1	1	2	2	1	5	1	7	1	3	5
Cabernet Sauvignon	3	5	5	1	3	5	5	3	7	3	1	3	3	2	2	3	5	1	5	1	3	7
Cardinal	2	2	5	1	1	5	3	5	3	1	9	1	3	3	2	1	1	1	1	1	1	3
Carrasquín	3	3	7	2	1	5	5	5	7	3	1	1	3	2	2	7	3	3	3	3	1	5
Chasselas Doré	3	3	5	1	3	5	5	5	3	3	1	2	3	2	1	1	5	1	5	1	3	5
Chasselas Rosé	3	3	5	1-2	1	5	5	5	3	3	1	1	1-3	2	1	1	1	1	3	1	1	5
De José Blanco	3	3	5	2-3	1	5	5	5	3	3	1	1	1	2	1	1	3	1	3	1	3	5
Doña Blanca	3	3	5	1	3	5	5	3	3-5	1	1	2	3	2	1	5	5	1	5	1	1	5
Furmint	3	2	5	1	3	5	5	5	3	3	1	1	1	3	1	3-5	5	1-5	3	1	1-5	3
Garnacha Tintorera	4	2	7	1	1	4	3	2-5	3	3	1	1	1	3	1	5	3	5	5	1	1	1
GEN 01	4	3	5	1	3	5	5	5	3	3	1	1	3	2-3	1	3	1	1	1	1	1	3
GEN 05	2	3	5	1	1	5	5	5	3	3	1	1	3	3	1	1	1	1	1	1	1	3
GEN 06	3-4	3	5	3	1-5	5	3	3	3	3	1	2	1-3	2	1	3	5	1	5	1	1	5
GEN 08	3	3	5	2-3	3	5	5	5	7	3	1	1	1-3	2	1	1	1	3	1	1	1	5
GEN 10	2-3	3	5	1	3	2-5	5	3	3	3	1	1	3	2	2	5	5	1	1	1	1	5
GEN 12	2	2	5	1	3	5	3	5	3	3	1	1	1	3	1	1	1	1	5	1	1	1
GEN 13	2	3	5	1	5	2	3	2	3	3	1	1	1-3	3	1	1	1	1	1	1	1	3
GEN 14	2	3	5	1	3	5	5	3	3	3	1	1	3	2	1	1	1	1	1	1	1	5
GEN 16	3	3	3	1	3	4	5	2	3	3	1	1	1	2	2	5	1	3	1	1	1	5
GEN 19	3	3	5	2	5	5	5	3	7	3	1	1	3	2-3	2	3	3-5	1	1	1	1	5
GEN 20	3	3	5	1	5	5	7	5	7	3	9	1	1	3	2	5	7	3	1	1	1	3-5
Godello	3	2	5	1	1	5	5	3	3	3	1	1	1	2	1	3-5	3	1	5	1	3	5
Italia	2-3	3	5	1	3	5	3	2	3	3	1	1	3-4	3	1	1	3	1	1	1	1	5
Lairén	3	3	5	1	3	5	5	5	5	3	9	1	3	2	1	5	5	3	5	1	1	5
Mazuelo	3	3-4	5	1	5-7	5	5	5	7	3	1	1-2	3	2	2	5	7	1	7	1	3	7
Mencía	2	3	5	1	1	5	3	3-5	3	3	1	1	1	3	1	1	1	1	1	1	1	5
Morenillo II	3	3	5	1-4	3	5	5	5	3	3	1	1	3	2	1	5	7	3	5	1	3	5
Morrastel Bouschet	3	3	7	3	1	5	3	5	3	3	1	1	3	2	1	5	5	3	3	3	3	5
Moscatel Blanco	3	2	5	1	1	5	5	2	5	3	1	1	3	2-3	1	1	3	1	5	1	1	5

Table 7. Values obtained for each descriptor of bunch and berry.

Cultivar	Bunch and berry																
	OIV 202	OIV 203	OIV 204	OIV 206	OIV 207	OIV 208	OIV 209	OIV 220	OIV 221	OIV 222	OIV 223	OIV 225	OIV 226	OIV 227	OIV 231	OIV 236	OIV 238
Albarín Blanco	3	5	5	3	1	2	2	5	5	2	3	1	2	7	1	2	3
Albarín Tinto	3	3	7	1	5	1	2	3	3	2	3	6	2	9	1	1	3
Aramon	5	5	5	5	1	1	2	5	5	2	2	5	2	7	1	1	3
Cabernet Sauvignon	3	3	3	3	5	1	2	3	3	2	3	6	2	9	1	4	1
Cardinal	9	5	3	3	1	1	2	7	7	1	3	5	1	7	1	1	7
Carrasquín	3	5	7	3	5	1	2	5	3	2	2	6	2	9	1	1	3
Chasselas Doré	5	3	5	3	1	1	2	5	5	2	2	1	2	5	1	1	3
Chasselas Rosé	3	3	5	3	0	1	2	5	5	2	2	2	2	5	1	1	3
De José Blanco	5	3	5	1	0	1	2	5	5	2	2	1	2	5	1	1	1
Doña Blanca	5	5	5	3	0	1	2	5	5	1	8	1	2	7	1	1	3
Furmint	3	3	5	1	0	1	1	3	3	2	3	1	2	7	1	1	3
Garnacha Tintorera	3	5	3	3	1	2	2	5	5	2	2	5	2	5	7	1	3
GEN 01	3	3	5	3	1	1	1	5	5	2	2	1	2	5	1	1	3
GEN 05	9	7	3	5	1	1	4	5	5	1	4	1	2	7	1	1	3
GEN 06	3	3	5	1	1	1	1	5	5	2	2	1	2	5	1	1	1
GEN 08	3	3	5	3	5	1	2	5	3	2	3	6	2	7	1	1	3
GEN 10	3	3	7	3	5	1	2	3	3	2	3	6	2	7	1	1	3
GEN 12	3	3	5	3	1	1	2	3	3	2	2	5	2	7	1	1	3
GEN 13	3	3	5	3	1	1	1	3	3	2	2	5	2	7	1	1	1
GEN 14	3	3	5	3	1	1	1	3	3	2	2	5	2	7	1	1	1
GEN 16	3	3	3	3	1	1	2	5	5	1	3	3	2	7	1	2-3	3
GEN 19	3-5	3-5	-	5	1	1-2	2	7-9	5-7	-	4	1	2	5	1	1	3
GEN 20	3	3	-	1-3	1	1	2	3	3	-	3	1	2	5	1	1	3
Godello	3	3	7	3	1	1	1	5	3	2	4	1	2	7	1	1	3
Lairén	3	3	3	3	1	1	1	5	5	2	2	1	2	7	1	1	3
Mazuelo	3-5	3	-	3	1	1	1-2	5	5	-	3	6	1	7	1	1	3
Mencía	5	5	7	3	1	1	2	5	5	2	3	6	2	9	1	1	1
Morenillo II	5	5	5	1	1	1	3	3	3	1	2	5	1	7	1	1	1
Morrastel Bouschet	5	5	5	3	1	3	2	5	5	2	2	6	2	7	7	4	3
Moscatel Blanco	5	3	7	3	1	1	2	3	3	2	2	1	2	7	1	2	1
Moscatel Rojo	5	3	7	1	1	1	2	3	3	2	2	3	2	7	1	2	1
Moscatel de Alejandría	5-7	3-5	5	3	0	1	2-3	5-7	5	1	3	1	2	7-9	1	2	3
Mouratón	3	5	7	1	1	1	2	5	5	2	3	5	2	7	1	1	3
Palomino	9	7	7	3	1	2	2-3	5	5	2	2	1	2	5	1	1	3
Petit Bouschet	1	3	3	3	1	2	2	3	3	2	2	6	2	9	9	1	3
Roseti	7	5	5	5	1	1	2	7	5	1	3	1	2	5	1	-	3
Savagnin Blanc	3	3	5	1	1	1	2	3	3	1	3	1	2	7	1	1	1
Sumoll	5	5	5	3	5	2	3	3	3	1	3	5	1	7	1	1	3
Verdejo Tinto	3	3	9	3	1	1	1	5	5	2	3	6	2	9	1	1	1

Taking into account the ampelographic and microsatellite data, as well as the information supplied by the vine growers, some of the cultivars cited in old references could be assigned to some of the unidentified ones, others were clones of different cultivars, and others were misidentified (Table 8).

DISCUSSION

In recent years, the survey and collection of cultivars in danger of extinction is being performed worldwide to safeguard existing grapevine genetic resources. Some of these cultivars can have considerable importance in parentage analysis or for breeding purposes. For instance, Gouais Blanc, a very old and nearly extinct cultivar that produces a poor quality wine, is the parent of well-known cultivars such as Aligoté, Aramon, Chardonnay, Furmint, Gamay Noir, Melon and Riesling (Bowers *et al.*, 1999a; Boursiquot *et al.*, 2004; http://www.vivc.de/). In addition, wine market trends are changing, with consumers demanding new styles of wine that can be provided by high quality minor cultivars.

The drastic reduction in vineyard lands that Asturias has experienced in the last century has led to considerable genetic erosion. The aging of local vine growers and the plantation with a limited number of cultivars approved by the *Cangas Quality Wine* appellation further accentuate this problem. Aware of the importance of preserving grapevine genetic resources, in 2005 SERIDA launched surveys in boroughs where grapevines were traditionally cultivated, with the aim of locating and identifying the full range of phenotypic diversity. Areas used for grape growing in the past (Feo, 1986; Fernández, 2004) were also surveyed, locating only isolated vines growing on trees, which were impossible to access, or on the verandas of houses, which were not pruned or were seriously affected by fungal diseases, thus making their study unreliable.

The cultivars characterized in this study were genotyped using microsatellite loci and ampelographically described *in situ* to confirm the molecular identification. Some of the cultivars (GEN 19, GEN 20, Roseti and Italia) could not be completely described because of problems in the fruit set, fungal diseases, or viruses that affected the production of the plant. Autochthonous, Spanish and foreign cultivars were found, as well as unknown cultivars not described to date. Most of the identified genotypes have also been found in the neighbouring regions of Galicia and Castilla-León (González-Andrés *et al.*, 2007; López *et al.*, 2009; Díaz-Losada *et al.*, 2010; Santana *et al.*, 2010).

A cluster analysis was subsequently performed using the model description of each cultivar. In the dendrogram thus obtained, Albarín Blanco and Savagnin Blanc are grouped together with a similarity

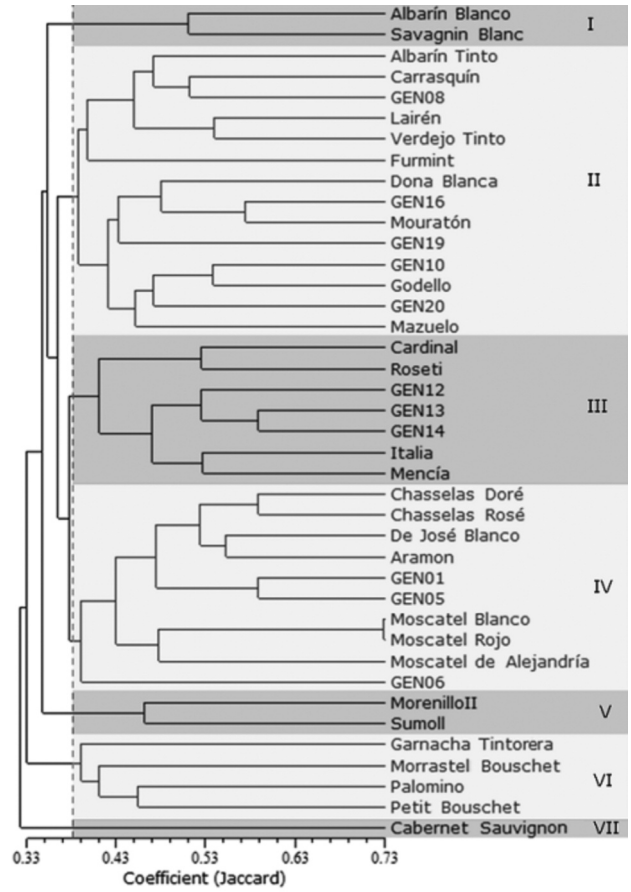

Figure 2. Dendrogram generated by applying the UPGMA method to the ampelographic data of the cultivars.

coefficient of around 0.52. In fact, vine growers give the former name to both cultivars. Albarín Blanco is an autochthonous cv. with muscat taste scarcely cultivated in north-western Spain and presenting a high quality for winemaking (Santiago *et al.*, 2005). Savagnin Blanc is probably a parent of the cultivar Trousseau Noir (http://plantgrape.plantnet-project.org/cepage/Trousseau % 20N), the last one being cultivated under the name Verdejo Tinto in Asturias.

Among other cvs., Group II includes Carrasquín, Albarín Tinto, GEN 08, GEN 10 and Verdejo Tinto, which also were clustered in a same group in the molecular study carried out previously (Moreno-Sanz *et al.,* 2011). Based on the evidence of microsatellite markers, Díaz-Losada *et al.* (2010 and 2012) suggest that Verdejo Tinto (synonym of Trousseau Noir) may well be an ancestor of some of the cultivars grown in north-western Spain studied in this paper (Albarín Tinto, Carrasquín, Albarín Blanco, Mencía, Godello, and Mouratón). Moreover, Albarín Tinto is considered one of the parents of Mouratón (Vitis International Variety Catalogue, http://www.vivc.de/). GEN 16,

Table 8. Cultivars cited in the past with their current correspondence.

Cultivar cited in old references	Correspondence/current name
Alvarín Negro	Albarín Tinto/Albarín Negro
Negrín	Albarín Negrín (clone of Albarín Tinto with small berries)
Pata de Perdiz	Mencía Pata de Perdiz (clone of Mencía with red peduncle)
Carrasco	Probably a clone of Carrasquín
Moscatel	Moscatel Blanco de grano menudo
Alicante	Probably Garnacha Tintorera
Jaén – Moscatel	Probably GEN 16
Verdeja	Probably GEN 01
Teta de vaca	Teta de Vaca (erroneous name for Moscatel de Alejandría cv.)
Pedro Jiménez	Pedro Jiménez (erroneous name for Palomino cv. in Illano)
Negrón	Currently there is a cv named Negrona (corresponding to Mouratón) that could be Negrón cited in the past
Carrascón	
Conrasión	
Garnacha Roja	
Malbec	
Mallén	Not found
Pardusco Prieto	
Picudo	
Bondal	
Rondales	
Rondal Negro	

with a foxy and muscat taste, is also found in this Group. It is called Jaén by some vine growers and Moscatel by others. Based on the ampelographic data obtained in this study, GEN 16 seems to be the Jaén cv. described in Asturias by Martínez and Pérez (1999). These authors suggested that Jaén might be a hybrid produced after the phylloxera plague from crossbreeding between *Vitis labrusca* and Moscatel; *Vitis labrusca* flavours are described as foxy (Cangi *et al.*, 2006). Naredo (1914) mentioned, but did not described, the Jaén-Moscatel cultivar, which might be the same Jaén described in our study due to the similarity in the name, although Naredo considers it a pre-phylloxera cultivar. GEN 19 is also included in this group. It has 15 out of 16 alleles in common with Italia and its ampelographic description matches with that of Italia in the Spanish Registry of Commercial Varieties (Chomé *et al.*, 2003).

Group III includes three cultivars (Cardinal, Roseti, Italia) used both as wine and table grapes (http://www.vivc.de/), another three (GEN 12, GEN 13, GEN 14) called "Productora" by the local vine growers (the name used for hybrids in the region) and Mencía. This grouping was not observed when using the previously analysed microsatellite markers, although Roseti is a grandparent of Cardinal (http://www.vivc.de/). It is worth noting that the only Cardinal accession studied had half of the young shoot tips half open, although this characteristic is described as fully open for this cultivar (Chomé *et al.*, 2003). Regarding Mencía, this variety is widely grown in north-western Spain, although it is of unknown origin (Martínez *et al.*, 2006). It was first cited in the "*Libro y registro de la bodega*" ("Book and register of the wine cellar") of the Guadalupe Monastery in Cáceres in 1520 (Villegas, 2007).

The Moscatel and Chasselas cvs., used both as wine and table grapes, are included in Group IV, together with De José Blanco (a hybrid), Aramon and three unknown cultivars (GEN 01, GEN 05 and GEN 06). Moscatel Rojo and Moscatel Blanco have the same microsatellite profile, as it is the case between Chasselas Rosé and Chasselas Doré, which can only be distinguished by the colour of the berry. Analysis using nine microsatellite markers (Moreno-Sanz *et al.*, 2011) clustered the Chasselas group with De José Blanco, and GEN 01 with GEN 06. GEN 01 was called Verdello Blanco by two old vine growers. Furthermore, one of these growers pointed out that it was one of the oldest cultivars in the region along with Albarín Blanco, but that it was no longer cultivated because of its high sensitivity to botrytis. This cultivar was previously described by Martínez *et al.* (2002). GEN 05 is a table grape cultivar. Only two accessions of GEN 06 were found; these plants had problems with berry set every year, which might be the underlying reason why this cultivar is nearly extinct.

Despite the presence of Palomino (a white cultivar), the ampelographic approach successfully grouped the teinturier cultivars in Group VI. Teinturier cultivars were also clustered using microsatellite markers, as Petit Bouschet is the parent of Morrastel Bouschet and Garnacha Tintorera (Alicante Henri Bouschet) (http://www.vivc.de/).

Some of the cultivars cited in old references have disappeared or may correspond to some of the unknown cultivars described in this work. Teta de Vaca and Pedro Jiménez are erroneous denominations of Moscatel de Alejandría and Palomino, respectively. Ten cultivars were not found in this work, among which Malbec and Garnacha Roja, which are cultivated in other Spanish regions. Finally, the Carrasquín analysed in a previous study (Moreno-Sanz *et al.*, 2011) presented the same genotype as the Carrasco analysed by Gago *et al.* (2009). However, some differences have been found between the Carrasco description performed by Martínez and Pérez (2000) and our description of Carrasquín (differences in the shape of the leaf, the teeth and the lateral upper sinus). In fact, Suárez (1879), on the basis of ampelographical descriptions, considered them as two different cultivars. Taking into account all this information, Carrasco and Carrasquín are probably different clones of the same variety with slight phenotypic differences between them.

CONCLUSION

The genetic erosion of grapevine resources worldwide has led in recent years to efforts to locate, identify, study and conserve cultivars at risk of extinction. In this respect, identification using microsatellite markers supplemented with ampelographic description is necessary to confirm the identity of cultivars and hence to improve the management of germplasm banks.

Great grapevine diversity was found in Asturian vineyards. Most of the unknown cultivars surveyed (GEN 01, GEN 05, GEN 06, GEN 08, GEN 10, GEN 12, GEN 13, GEN 14 and GEN 20) are in danger of extinction, as shown by the small number of accessions (less than ten) found of each cultivar. These cultivars have been described here for the first time. Some of them are hybrids ; others may be closely related to autochthonous cultivars. Being aware of the risk of these cultivars disappearing, vegetal material has been sent to the El Encín National Germplasm Bank (Madrid, Spain) for conservation and future study. These cultivars could be of major importance in the future for potential breeding or technological purposes.

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