

## Identification of autochthonous grapevine varieties in the germplasm collection at the ITA of 'Castilla y León' in Zamadueñas Station, Valladolid, Spain

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### Abstract

During the last decades a study of the grapevine collection located at the Zamadueñas Station of the *Instituto Tecnológico Agrario de Castilla y León* (ITACyL) was carried out. Recently, molecular markers have been used for varietal identification and detection of the possible synonymies in the collection. This work shows the results of that characterisation, as well as the detected synonymies and homonymies in the study. The maintenance of the existing plant material is recommended, particularly with reference to the varieties with a marked risk of extinction because they are not presently object of new plantations.

**Additional key words:** ampelography, germplasm bank, microsatellites, molecular markers, STMS.

### Resumen

#### Identificación de variedades autóctonas de vid en la colección del ITA de Castilla y León en la Estación de Zamadueñas, Valladolid, España

Durante las últimas décadas se ha llevado a cabo un estudio del material vegetal existente en la colección de variedades de vid de la finca de Zamadueñas, del Instituto Tecnológico Agrario de Castilla y León (ITACyL). Recientemente, se han utilizado marcadores moleculares, con el objetivo de identificar y detectar posibles sinonimias existentes en dicha colección. En el presente trabajo se exponen los resultados de dicha caracterización, así como la relación de sinonimias y homonimias detectadas en el estudio. Se concluye con la recomendación del mantenimiento del material existente, particularmente las variedades que se encuentran en marcado peligro de desaparición por no ser objeto de cultivo en la actualidad.

**Palabras clave adicionales:** ampelografía, banco de germoplasma, marcadores moleculares, microsatélites, STMS.

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### Introduction

At the present time there are at least ten ampelographic collections of grapevine in Spain plus the *Vitis* germplasm bank located at 'El Encín' (Alcalá

de Henares, Madrid), all of them aiming to conserve the grapevine variability existing in the country (Hidalgo, 1999). The increasing importance of the Denominations of Origin (DO) in Spain, each one including a reduced number of authorised varieties, marks a tendency to the reduction of variability in the grapevine growing regions.

The grapevine collection located at the *Instituto Tecnológico Agrario* (ITACyL) of Castilla y León in

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Received: 23-09-05; Accepted: 30-11-05.

Zamadueñas (Valladolid) includes a total of 162 accessions, 30 table grapes and 132 wine grape varieties (Hidalgo, 1999). Forty one of them are autochthonous cultivars that were collected in the region in different prospecting missions carried out in the last decades. These accessions were introduced with the varietal name that was used in their sampling sites. Preliminary morphological characterisation of the plants pointed to possible homonymies or synonymies among the collected plant material. Molecular characterisation of these plants has been carried out in order to detect or confirm the true to typeness of the plants and complete the varietal identification of each accession.

García de los Salmones (1914) mentioned in the two regions corresponding to the present day *Castilla y León* political division, a total of 162 white and 175 red varieties, although this number very likely includes a high number of synonymies, some of them already detected. Nevertheless, the evolution of the viticulture in the region, with a growing number of plantations and with a reduced number of authorised varieties, is leading to the loss of a high part of the varietal patrimony.

The germplasm bank of the *Comunidad de Madrid* (BGVCAM) contains 130 accessions from the mentioned region (Cabello, 1995), that include a part of the existing variability of the zone.

The increasing interest in avoiding the loss or extinction of grapevine varieties and the complete identification of the already collected material has motivated the present work, the objective of which is to detect the existing synonymies as well as to identify in all possible cases the correspondence of the accessions to other known varieties.

## Material and Methods

Table 1 lists all the studied accessions, with the indication of the colour of the berry and their name in the respective places of collection. Some accessions were named when introduced in the collection, and in some other cases a second name or a number was assigned because of the presumed existence of a homonymy. A minimum of eight plants at a 2.8 × 1.4 m spacing were planted in the collection plot, in a sandy clay loam soil. Cultural practices were the traditional in the zone.

The identification of the studied accessions was carried out on the basis of the molecular analysis, complemented with the ampelographic observations, made on each accession following the *Organisation Internationale de la Vigne et du Vin* (OIV, International Organisation of Vine and Wine) descriptors, and comparing with the existing databases (Martín *et al.*, 2003). The last column in Table 1 indicates the varietal names for each accession.

Sampling for molecular analysis consisted of young fresh leaves that were collected in the field and kept fresh or after lyophilization at -80°C until analysed.

DNA extraction and amplification were carried out by using the MasterPure™ Plant Leaf Purification Kit (Epicentre Technologies, Madison Wis.), and the following six STMS loci were used: VVS2 (Thomas and Scott, 1993), VVMD5 and VVMD7 (Bowers *et al.*, 1996), and *ssrVrZAG47*, *ssrVrZAG62* and *ssrVrZAG79* (Sefc *et al.*, 1999), under the conditions detailed in a previous work (Martín *et al.*, 2003). Polymorphism of the amplified products was detected in an automated DNA sequencer ABI PRISM model 310 (PE Applied Biosystems). As a result of the analysis, genotypes for each variety were obtained for the studied loci.

## Results

The following identification results were obtained:

### Major varieties

1) *Temprano blanco*, is a variety known under this name in some places like Cigales (Valladolid), and under the name *Malvasía* in the DO 'Bierzo'. It is the French '*Chasselas doré*' (<http://www.imianet.org/exploraelencin/web/003/>), also collected in Spain with the names *Temprana Agosteña* (CyL-19), *Temprana Media*, *Temprana Tardía*, *Tempranillo de Nava*, *Albillo Negro* and *Elba*. It is present in many places of the region, frequently designed with names that include the term 'Temprano' or 'Temprana', that means early.

2) *Tinta del País* is the *Tempranillo*, an already known synonymy. It is also synonymous with *Tinta de Nava*, that is a red variety included in the collection with the name of the locality of sampling. A grey berry mutation is the *Tinta de Toro Blanca*, the name of which comes the light colour of the berry; it should be

**Table 1.** Plant material studied and varietal identification

Accession number	Berry colour <sup>1</sup>	Accession name <sup>2</sup>	Zone of origin	Varietal identification <sup>3</sup>
CyL-01	B	Albillo Nava	Rueda	Verdejo
CyL-02	B	Albillo Negro	Cigales	Temprano Blanco
CyL-03	B	Alcazpepita	Rueda	Cañorrojo
CyL-04	B	Calagraña	Rueda	Calagraña
CyL-05	B	Doradilla 1	Rueda	Doradilla
CyL-06	B	Doradilla 2	Rueda	Unknown
CyL-07	B	Elba	Toro	Temprano Blanco
CyL-08	B	Hornipepita	Rueda	Cañorrojo
CyL-09	B	Huerta del Rey	Cigales	Marfal
CyL-10	B	Marta Nava	Rueda	Salvador
CyL-11	B	Moscatel de Toro	Toro	Moscatel de Grano Menudo
CyL-12	B	Pirulés Dorada	Ribera del Duero	Malvasía Riojana
CyL-13	B	Pirulés Verde	Ribera del Duero	Malvasía Riojana
CyL-14	B	Prieto Picudo Blanco 1	Tierras de León	Godello
CyL-15	B	Prieto Picudo Blanco 2	Tierras de León	Godello
CyL-16	B	Puente	Toro	Salvador
CyL-17	B	Rojal	Ribera del Duero	Malvasía Riojana
CyL-18	B	Temprana Agosteña 1	Cigales	Lairén
CyL-19	B	Temprana Agosteña 2	Cigales	Temprano Blanco
CyL-20	B	Temprana Media	Cigales	Temprano Blanco
CyL-21	B	Temprana Tardía	Cigales	Temprano Blanco
CyL-22	B	Tempranillo de Nava	Rueda	Temprano Blanco
CyL-23	B	Tolociriana	Rueda	Castellana Blanca
CyL-24	B	Verdegudillo	Cigales	Doña Blanca
CyL-25	G	Tinta Toro Blanca	Toro	Tempranillo Gris
CyL-26	R	Juliana	Rueda	Juliana
CyL-27	N	Cenicienta	Rueda	Cenicienta
CyL-28	N	Mencia Pajalal	Bierzo	Mencia
CyL-29	N	Negrera	Bierzo	Juan García
CyL-30	N	Pan y Carne 1	Bierzo	Merenzao
CyL-31	N	Pan y Carne 2	Bierzo	Pan y Carne
CyL-32	N	Prieto Picudo Oval 1	Tierras de León	Prieto Picudo
CyL-33	N	Prieto Picudo Oval 2	Tierras de León	Prieto Picudo
CyL-34	N	Rufete Vi	Sierra de Francia	Rufete
CyL-35	N	Rufete Mi	Sierra de Francia	Rufete
CyL-36	N	Rufete Se	Sierra de Francia	Rufete
CyL-37	N	Tinta Madrid	Cigales	Bobal
CyL-38	N	Tinta de Nava	Rueda	Tempranillo
CyL-39	N	Tinta del País	Ribera del Duero	Tempranillo
CyL-40	N	Verdejo Tinto	Rueda	Puesto Mayor
CyL-41	N	Villarino	Arribes del Duero	Juan García

<sup>1</sup> B = white; G = grey; R = red; N = black. <sup>2</sup> Name of the accession when introduced in the collection. <sup>3</sup> Proposed name after comparison with existing databases and germplasm collections.

more convenient to call it *Tempranillo Gris* or *Tinta de Toro Gris*.

3) *Albillo de Nava* corresponds to the variety *Verdejo*. In fact, the name *Albillo* includes several cases of homonymy, none of which had so far been related with *Verdejo*. It is recommended not to use in this case the name *Albillo*, to avoid confusion.

4) Several accessions of the varieties *Rufete* and *Prieto Picudo* have been detected, that could be clones with some intravarietal variability. It also was in the collection a clone of *Mencia*, with rather short internodes, with the name *Mencia pajalal*.

5) The variety *Juan García*, grown in the 'Arribes del Duero' has been collected in this region as *Negrera*

and also *Villarino*, this last name probably due to its geographical origin in a locality of the 'Arribes del Duero'. The same variety is called *Mouratón* or *Negreda* in the DO *Ribeira Sacra* and in other zones of Galicia.

6) Some other known varieties have been detected under different names: *Merenzao* as *Pan y Carne* (CyL-31), which is a homonymy leading to errors existing in different places of the region, particularly in El Bierzo; *Godello*, with the name of *Prieto Picudo Blanco*; *Doña Blanca* as *Verdegudillo*, and *Bobal* as *Tinta Madrid*.

7) *Malvasía Riojana*, a synonymy of *Alarije* from Extremadura, *Subirat Parent* from Catalonia and *Blanquirroja*, *Rojal* or *Tobia* from La Rioja (Rodríguez-Torres *et al.*, 2000), has been collected with the name *Pirulés* and *Rojal*, being the last one a homonymy also used for other varieties.

#### Minor varieties

Some of the accessions have been identified as synonymies of known varieties while others were not previously described. The varieties included in this group have a more or less marked risk of extinction, since only isolated plants were reported in most of the cases.

1) *Cañorrojo*, a variety existing in the BGVCAM at 'El Encín', was collected with the names *Alcazpepita* and *Hornipepita*.

2) *Doradilla* (CyL-05) was collected with this name. But also plants not corresponding to any known variety have been collected under the same name.

3) *Castellana Blanca* was collected as *Tolociriana* and *Lairén* as *Temprana Agosteña* (CyL-18).

4) *Huerta de Rey*, also existing in the BGCAM at 'El Encín', is a synonymy of the variety *Marfal* from Extremadura.

5) *Puesto Mayor*, a synonymy of the portuguese variety *Saborinho*, has been localized with the name *Verdejo Tinto*.

6) Two accessions localized at Nava del Rey and Toro, *Marta Nava* and *Puente* correspond to the variety *Salvador*, also existing in the BGCAM at 'El Encín'.

#### Endangered varieties

Finally, four of the accessions correspond to varieties from which no previous written references or

plant material in other grapevine collections have been found. Consequently they are in a marked risk of extinction. They are: 1) *Calagraña*, a white wine variety; 2) *Pan y Carne* (CyL-31) and *Cenicienta*, two red wine varieties, and 3) a table grape variety known as *Juliana*, with red berries. This variety was previously named *Jami* by Rojas Clemente (2002 facsimile edition, original of 1807), and it has been recently identified in the collection of the 'Jardín Botánico de Madrid' (F. Cabello, personal communication).

Table 2 shows the obtained genotypes for each variety. Any of them can be distinguished from the others with the exception of *Tempranillo* and *Tempranillo Gris*, that have the same genotype for these loci, since the last one is considered as a mutation of *Tempranillo* that affects the colour of the berry. In the rest of the cases, any two varieties can be distinguished with only four microsatellites.

The percentages of homozygosity oscillate between 3.8 to 23.1%, with an average of 14.7%. The total number of genotypes ranges from 11 to 17 with an average of 14.5, and the number of alleles from 6 to 10 with an average of 8 (Table 3).

As it can be observed in Table 2, there are 10 varieties in a marked risk of extinction, since only isolated plants have been detected, no new plantations are established with them, and no plant material for propagation is available in commercial nurseries.

## Discussion

The six SSR markers used here have been established for characterisation and identification of grapevine varieties in a broad European *Vitis* germplasm database (<http://www.genres.de/eccdb/vitis/>), and they are able to uniquely identify most of the grapevine varieties. For this reason, they have been widely used in genotype characterisation, mainly among *Vitis vinifera* collections of cultivars (Vignani *et al.*, 1996; Sefc *et al.*, 1998; Lopes *et al.*, 1999; Sánchez-Escribano *et al.*, 1999; Sefc *et al.*, 2000; Martín *et al.*, 2003).

With respect to the best known varieties, it is advised to reduce the use of local synonymies in order to avoid confusion in the identification of the varieties. In some cases, the most usual name of the variety depends on the zones, as it is the case of *Juan García* or *Mouratón*,

**Table 2.** Genotypes of the studied varieties for the six used STMS loci

Berry colour	Variety	STMS loci					
		ZAG47	VVMD5	ZAG62	VVMD7	VVS2	ZAG79
B	Calagraña <sup>1</sup>	FG	HH	BC	BE	BE	CH
B	Cañorrojo <sup>1</sup>	DG	FH	AB	BB	EH	FH
B	Castellana Blanca <sup>1</sup>	DE	BF	AA	BB	DH	FF
B	Doña Blanca	CC	AE	AF	BE	CG	EE
B	Doradilla	CE	FG	BB	BC	DE	AE
B	Godello	EF	BG	AB	BC	GH	FF
B	Lairén	EG	CG	AC	BD	AE	BH
B	Malvasía Riojana	EG	EF	AB	BB	DE	FH
B	Marfal	EG	CE	BD	BB	CE	AH
B	Moscatel de grano menudo	BG	CF	AD	AE	AA	FG
B	Temprano Blanco	EF	CF	CF	BD	AD	FI
B	Unknown <sup>1</sup>	BG	DH	BE	BG	AE	FH
B	Verdejo	DF	BG	AC	BI	GH	FF
G	Tempranillo Gris <sup>1</sup>	DD	FF	DE	BG	DE	EF
R	Juliana <sup>1</sup>	EG	BC	BF	BB	CF	EH
N	Bobal	CF	CE	BB	BC	EF	CE
N	Cenicienta <sup>1</sup>	BD	BF	EF	DG	AD	FF
N	Juan García	CF	EG	BF	EI	CG	EF
N	Mencia	CF	BF	BC	EI	EG	EH
N	Merenzao	AF	GG	BB	BI	DG	DE
N	Pan y Carne <sup>1</sup>	CF	BG	CE	GI	DG	DJ
N	Prieto Picudo Tinto	BF	BG	BC	BI	DG	FF
N	Puesto Mayor <sup>1</sup>	CF	BG	BC	BI	AG	DJ
N	Rufete	CF	BF	BC	BI	AH	DE
N	Salvador <sup>1</sup>	BF	FH	BE	BF	BE	CF
N	Tempranillo	DD	FF	DE	BG	DE	EF

<sup>1</sup> Varieties with a high risk of extinction.

**Table 3.** Genotype variability in the analysed STMS loci

	Average	STMS loci					
		ZAG47	VVMD5	ZAG62	VVMD7	VVS2	ZAG79
Homozygosity percentage	14.7%	11.5%	15.4%	15.4%	19.2%	3.8%	23.1%
Number of different genotypes	14.2	14	15	14	11	16	15
Number of obtained alleles	8.0	7	8	6	9	8	10

*Tinta del País* or *Tempranillo* and some others already mentioned in the Results section. The use of homonymies should also be avoided as much as possible, since they frequently lead to errors in the identification of the varieties. This is the case of *Albillo* among others.

The identification of minor varieties already described and existing in some germplasm bank (Cabello, 1995) is important, since the documentation

of this bank indicates that those accessions come from the same geographic region. This is the case of *Cañorrojo*, *Huerta de Rey*, *Puesto Mayor* and *Castellana Blanca*. These accessions should be studied in order to explore the intravarietal variability for propagation or reintroduction purposes in the region.

There are also some varieties that are in a marked risk of extinction, for which no previous information has been found. These are: i) *Calagraña*, from Rueda;



ii) the unknown variety that was introduced with the name *Doradilla 2* (Table 1), a name that should be changed to avoid the existing homonymy (*Doradilla 1*); iii) the mutation *Tempranillo gris*; and iv) *Pan y Carne* that is considered as the true to type variety (CyL-31, Table 2). All these varieties should be thoroughly characterised. New prospectations are under way in order to collect and maintain a maximum of variability for further propagation.

With respect to the genotype variability (Table 3), the homozygosity percentages roughly agree with those obtained in a previous study with more than 300 Spanish varieties (Martín *et al.*, 2003). The number of alleles and genotypes that were obtained was lower than the ones obtained in the mentioned study, as expected by the much smaller number of studied accessions.

As a conclusion of the present work, it is recommended to fully characterize each of the varieties, mainly the ones in risk of extinction. Also the collection of a maximum of intravarietal variability is recommended. The maintenance of this material for future use or reintroduction is of high interest.

## Acknowledgements

This work has been financially supported by the Spanish Ministry of Education and Science, under the research projects RF02-004-C5 and RF02-022-C1 and C2.

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