AMPELOGRAPHY - AN OLD TECHNIQUE WITH FUTURE USES: THE CASE OF MINOR VARIETIES OF *VITIS VINIFERA* L. FROM THE BALEARIC ISLANDS

Sonia GARCIA-MUÑOZ *, Gregorio MUÑOZ-ORGANERO,
María Teresa de ANDRÉS and Félix CABELLO

Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario (IMIDRA),
Finca El Elcín, 28800 Alcalá de Henares (Madrid), Spain

Abstract

Aims: We report the genetic, phenological, agronomic and ampelographic characterization of 27 minor grapevine accessions (*Vitis vinifera* L.) from the Balearic Islands (Spain). The influence of occasional climatic phenomena (hailstorm) and the ampelographer's experience on these characteristics was studied.

Methods and results: In 2006 and 2007, grapevine accessions were analyzed using 57 OIV (Office International de la Vigne et du Vin) qualitative and quantitative descriptors and six microsatellite loci (SSR). Ampelography is a good preliminary technique for the identification of plant material, since the ampelographic description carried out showed a high degree of similarity between the repetitions of one single accession (higher than 90%), confirming the microsatellite results. The colour of the young leaf's upper side (OIV-051), the juiciness of the flesh (OIV-232) and the firmness of berry flesh (OIV-235) were the most difficult characters to distinguish by ampelographers. In spite of the greater similarity found among the varieties studied, there were a few strong discriminant characters (OIV-225, OIV-084, OIV-053, and OIV-004). In addition, the ampelographic descriptions, agronomic parameters and phenology were influenced by hailstorm.

Conclusions: The combination of morphological and molecular characterizations of 27 accessions collected in the Balearic Islands (Spain) allowed their classification into 17 different vine varieties. The genetic analysis identified Beba blanca as a possible somatic mutant derived from Beba roja. The hailstorm increased the vegetative period and mostly affected mature leaves, bunches, agronomic characteristics and must composition.

Significance and impact of the study: The present work characterizes, for the first time, the ampelographic and molecular profiles of these minor varieties. It is provide agronomic information about old cultivars never published before which could be interesting for the grape growers.

Key words: morphology, grapevine, agronomic characterization, descriptors, hailstorm influence

Résumé

Objectifs: L'objectif de ce travail est d'évaluer les caractéristiques génétiques, phénologiques, agronomiques et ampélographiques de 27 accessions de vigne minoritaire (*Vitis vinifera* L.) des Iles Baléares (Espagne). L'influence de différentes variables agronomiques, de la grêle et de l'expérience des ampélographes a été étudiée.

Méthodes et résultats: Différents cépages ont été analysés en utilisant 57 descripteurs quantitatifs et qualitatifs OIV et six microsatellites. L'ampélographie est une bonne technique préliminaire pour connaître le matériel végétal, ont montré plus de 90% de similarité pour la même répétition des descriptions ampélographiques, confirmant les résultats de microsatellites. La couleur de la face supérieure de la jeune feuille (OIV-051), succulence de la pulpe (OIV-232) et la fermeté de la pulpe (OIV-235) étaient les caractères les plus difficiles à distinguer par les ampélographes. Malgré la grande similarité trouvée entre les échantillons, certains forts caractères sont importants pour identifier de ces variétés (OIV-225, OIV-084, OIV-053, OIV-004). De plus, les descriptions ampélographiques, les paramètres agronomiques et phénologiques ont été influencés par la grêle.

Conclusions: La combinaison des caractéristiques morphologiques et moléculaires de ces 27 cépages collectés aux Iles Baléares (Spain) a permis de les grouper en 17 variétés différentes de vigne. L'analyse génétique a montré que Beba blanca pouvaient être un mutant somatique de Beba roja. La grêle a augmenté la période végétative, a affecté particulièrement les feuilles matures, les grappes, la composition du moût et les caractéristiques agronomiques.

Signification et impact de l'étude: Pour la première fois ce travail a caractérisé des profils ampélographiques et moléculaires de ces variétés mineures. L'étude a montré le potentiel et l'intérêt des cultivars dont leur utilisation pourrait être importante pour les viticulteurs.

Mots-clés: morphologie, vigne, caractérisation agronomique, descripteurs, influence de la grêle

manuscript received 8th January 2010 - revised manuscript received 26th 2011

*Corresponding author: sonigarmu@gmail.com

J. Int. Sci. Vigne Vin, 2011, **45**, n°3, 125-137

©Vigne et Vin Publications Internationales (Bordeaux, France)

INTRODUCTION

Until a few years ago, ampelography was the main method used for describing and identifying vine varieties (*Vitis vinifera* L.; SCHNEIDER *et al*., 2008). Ampelographic studies using well defined OIV (Officine International de la Vigne et du Vin), UPOV (Union Internationale pour la Protection des Obtentions Végétales) and UPGRI (International Plant Genetic Resources Institute) official descriptors supplied insights into plant material identity (PAVEK *et al*., 2003). The careful notations on the phenotypic characteristics of different cultivars taken by important ampelographers over one hundred years ago (MARÉS, 1890-1891) have been confirmed years afterwards by genetics, as it is the case for the cultivar Muscat fleur d'oranger (SCHNEIDER *et al*., 2008), validating ampelography as a preliminary method for clarification plant material identity. However, in the last few years, confusing vegetable material due to a lack of ampelographic knowledge has led to legal and commercial controversies.

Recently, molecular markers, and specially microsatellites, have become an essential tool for the identification of grape varieties (THIS *et al*., 2004). This approach has the advantage of not being influenced by the environment or subjective interpretations, often attributed to some ampelographic descriptors, and it facilitates the exchange of results between laboratories by comparison of genetic profiles. However, differences based on somatic mutations such as density of prostrate hairs between main veins on lower side of blade in mature leaves or berry shape can only be certified if the material is evaluated in the field. Furthermore, the microsatellite technique is not adapted for clonal evaluation because it displays too low polymorphism (CABEZAS *et al*., 2003), in which case it is necessary to have the description of the varieties, given that the genetic analyses showed no difference from the original plants (BESSIS, 2007). For these reasons, molecular markers must be complemented by ampelography (CRESPAN *et al*., 2008; CUNHA *et al*., 2009).

The number of cultivated vine varieties has dropped in most European countries as most of the new plantations are based on varieties included under quality designations (Designations of Origin). As a result, many of the old indigenous varieties are at risk of becoming extinct (DE MATTIA *et al*., 2007). However, today´s wine consumers are demanding new products, which has increased the interest of winemakers and researchers for traditional old minor varieties (SANTIAGO *et al*., 2008). In this way, the use of traditional ampelography has re-emerged as the only way to verify whether the material found corresponds or not to the material quoted in the bibliography, by comparing the ampelographic descriptions (CERVERA *et al*., 2001).

The ampelographic and agronomic characterization of minor varieties is a pre-requisite for the establishment of a germplasm collection (ALLEWELDT and DETTWEILER, 1989). Simultaneously, ampelographic description is required for inscribing the varieties in the register of commercial varieties or in the variety catalogue (CHOMÉ *et al*., 2003; UPOV, 2007), before vine growers may use them. At the same time, the ampelographic technique in planted material is cheaper and more accessible than the microsatellites, which require specialised equipment. So, the morphological description of vine varieties could be used to prevent possible mistakes in plantations, nurseries or in vine growers, being later verified by the microsatellite technique. The combination of molecular markers and ampelographic descriptions irrefutably establishes varietal identity in a more thorough way and leads to more reliable and objective results (HINRICHSEN *et al*., 2001; SANTIAGO *et al*., 2005).

Some authors have suggested that atmospheric changes have an influence on morphological characteristics (ALLEWELDT and DETTWEILER, 1989; CUNHA *et al*., 2009) and that vineyard management creates changes in ampelographic descriptions (BOWERS *et al*., 1993). Because of that, morphological descriptions have been discredited and considered less reliable because they can be influenced by atmospheric changes and they may be the result of subjective evaluations (CERVERA *et al*., 2001). Knowledge of the factors that influence morphological descriptions, therefore, is an important requirement for developing a well defined ampelographic characterization. In this work, we have analyzed 27 grapevine accessions (*Vitis vinifera* L.) natives from the Balearic Islands (Spain), where no previous morphological studies had been performed. Ampelographic descriptions are the first step towards the identification and the full understanding of the agronomic and phenological development of these minor varieties. These varieties were collected from 1914 to 2005 in the Balearic Islands and then introduced in the *Vitis* Germplasm Bank (VGB) "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain), however, nowadays some of these varieties are no longer found in their original location. An accurate identification of these varieties is crucial to preserve the genetic variability, as well as to encourage their use and to provide new wine products to consumers.

For this purpose, we examined whether: (1) the 27 grapevine accessions differ among themselves by using morphological and genetic analysis, (2) the identification of the most influential descriptors and their relationships with the described varieties could facilitate field

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 1 – List of the 27 accessions used in this study and their profiles at six microsatellite loci.

References cultivars (Cabernet-Sauvignon, Chardonnay, Grenache (synonym Garnacha), Merlot and Mourvèdre) are included.

Local names [1]	Acc. [2]	Id. [3]	Berry colour	Use	Prime name	VVMD7 [4]		VVS2 [4]		VVMD5 [4]		VVMD27 [4]		VrZAG62 [4]		VrZAG79 [4]	
Batista	E23	BAT.E23	Black	Wine	BATISTA	237	251	141	151	229	235	179	189	188	200	245	251
Mateu*	E39	BEB.E39	White	Wine	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Corazón de Ángel**	O16	BEB.O16	White	Wine	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Valenci blanco**	O17	BEB.O17	White	Wine	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Grumiere blanco**	O44	BEB.O44	White	Wine	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Jaumes	E14	BEB.E14	White	Wine	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Calop	E32	BEB.E32	White	Wine	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Calop blanco	E43	BEB.E43	White	Table	BEBA	241	247	133	141	233	237	181	189	188	204	243	247
Beba negra, Calop negro, Calop negre	E18	BEN.E18	Black	Table, Wine	VALENCI TINTO	237	241	135	141	225	233	181	189	188	196	243	247
Calop rojo, Calop roig	E19	BER.E19	Rosé	Wine	BEBA ROJA	241	247	133	141	233	237	181	189	188	204	243	247
Vinaté, Vinater	E11	BOB.E11	Black	Wine	BOBAL	237	241	144	146	225	231	181	189	188	188	243	247
Boal	E21	BOB.E21	Black	Wine	BOBAL	237	241	144	146	225	231	181	189	188	188	243	247
Callet	E26	CAL.E26	Black	Wine	CALLET	237	247	131	141	233	237	181	189	188	196	243	247
Eperó de Gall	E37	EPE.E37	Black	Wine	EPERO GALL	241	247	131	144	223	231	179	181	188	204	243	257
Excursach	E27	EXC.E27	Black	Wine	ESCURSACH	237	237	141	144	223	237	181	181	188	196	247	251
Fogoneu	E24	FOG.E24	Black	Wine	FOGONEU	237	245	131	144	233	237	179	181	196	204	247	251
Mancés de Capdell	E31	GIR.E31	Black	Wine	GIRÓ	245	247	131	131	223	233	179	181	204	204	247	247
Giró	E36	GIR.E36	Black	Wine	GIRÓ	245	247	131	131	223	233	179	181	204	204	247	247
Gorgollasa, Gorgollassa	E25	GOR.E25	Black	Wine	GORGOLLASSA	237	247	141	151	219	237	179	194	188	204	257	261
Mansés de tibbus, Mancés de Tibbus	E30	MES.E30	Black	Wine	MANSÉS DE TIBBUS	241	247	131	151	219	223	181	181	188	204	243	243
Manto negro	E29	MTN.E29	Black	Wine	MANTO NEGRO	237	241	131	144	231	233	181	194	186	188	247	257
Cabellis	E20	MTN.E20	Black	Wine	MANTO NEGRO	237	241	131	144	231	233	181	194	186	188	247	257
Pampolat Girat***	H24	PAM.H24	Black	Wine	PAMPOLAT GIRAT	241	247	131	141	233	237	181	183	188	204	247	259
Pensal blanco, Moll, Prensal blanco	SCL	PEN.SCL	White	Wine	PENSAL BLANCO	237	241	135	141	231	231	179	194	188	196	249	257
Massacamps	E38	QUI.E38	White	Wine	QUIGAT	241	247	144	151	229	231	181	181	186	188	247	261
Quigat	E33	QUI.E33	White	Wine	QUIGAT	241	247	144	151	229	231	181	181	186	188	247	261
Sabaté, Sabater	E35	SAB.E35	Black	Wine	SABATÉ	237	241	131	131	225	231	185	194	188	196	237	257
Cabernet sauvignon	Reference		Black	Wine	CABERNET-SAUVIGNON	237	237	137	151	229	237	175	189	188	194	247	247
Chardonnay	Reference		White	Wine	CHARDONNAY	237	241	135	141	231	235	181	189	188	196	243	245
Grenache	Reference		Black	Wine	GRENACHE	237	241	135	144	223	237	194	194	188	188	257	257
Merlot	Reference		Black	Wine	MERLOT	237	245	137	151	223	233	189	191	194	194	259	259
Mourvèdre	Reference		Black	Wine	MOURVÈDRE	247	247	131	151	223	237	179	189	188	204	251	261

1 Local names are the names of the accession; 2 Accession (Acc.); 3 Code udes in the analysis (Id.); 4 SSR marker results correspond to size range (bp)

©Vigne et Vin Publications Internationales (Bordeaux, France)

descriptions, (3) occasional climatic phenomena (hailstorm) conditioned agronomic, ampelographic descriptions and phenology and (4) the ampelographer's experience and objectivity influences ampelographic descriptions, identifying the more difficult descriptors.

MATERIAL AND METHODS

1. Site description and climate data

The study was carried out at the *Vitis* Germplasm Bank (VGB) "Finca El Encín" (IMIDRA) located in Alcalá de Henares, Madrid, Spain (40º31´N, 3º17´W, 610 m asl, semiarid Mediterranean climate). The climate data were obtained from the meteorological station located at the VGB. Average precipitation (mm) and temperature (ºC) were compiled monthly over the study period (January 2006-December 2007), since certain climatic factors may interfere with ampelographic descriptions (ALLEWELDT and DETTWEILER, 1989). The 52-year mean monthly rainfall and temperature recorded at this station were also included as reference (period 1956-2007).

2. Plant material

The plant material described in this study corresponds to *Vitis vinifera* L. varieties introduced in the VGB "Finca El Encín" from the Balearic Islands, except the four accessions of the Beba variety (E39 from Gerona, and O16, O17 and O44 from Valencia, and Pampolat girat variety (H24) from Tarragona; table 1). The 27 different accessions were planted in 2002, all of which are grafted onto Richter 110 rootstock, trained to a simple cordon, pruned at eight buds per vine and were adult plants at the time of study. The planting density was 4444 vines/ha (0.90 m x 2.50 m).

3. Ampelographic description

Ampelographic descriptions were carried out during two consecutive years (2006 and 2007; UPOV, 2007), and old descriptions reported in 1998 and 1999 (stored in VGB) were used as reference. The observations were made according to OIV specifications (OIV, 1984), recording a total of 57 characters at specific phenological state and including both qualitative and quantitative characters (table 2). In 2006 were used the descriptors recommended by OIV (1984) modified by the GENRES 081 Proyect (European *Vitis* Database, www.genres.de /*vitis*/*vitis*.htm), but in 2007 we used the updated descriptors specified in the project GrapeGen 06 project (http://www1.montpellier.inra.fr/grapegen06/accueil.php). Therefore, it was necessary to standardize the 1998-1999 and of 2006 descriptions according to the 2007 descriptors. All varieties were described with a minimum of five plants (UPOV, 2007). A minimum of 10 young shoots, adult leaves, shoots, bunches and woody shoots were sampled per vine. For berry description, 30 berries belonging to the middle of ten representative bunches were sampled per vine. All varieties were described by three ampelographers with different degree of experience. Therefore, a minimum of 30 data were

Table 2 - Ampelographic characteristics according to the OIV descriptor list for grape varieties (OIV, 1984).

Parameters	Vine part	Descriptors
Qualitative	Shoot tip	OIV-001, ***OIV-002***, ***OIV-003***, ***OIV-004***
	Young leaf and shoot	OIV-007, ***OIV-008***, ***OIV-015-2***, OIV-016, ***OIV-051***, ***OIV-053***
	Woody shoot	**OIV-103**
	Flower	OIV-151
	Mature leaf	*OIV-067*, ***OIV-068***, ***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-087***, *OIV-094*, **OIV-306**
	Bunch	*OIV-204*, ***OIV-208***, ***OIV-209***
	Berry	OIV-220, **OIV-221**, ***OIV-223***, ***OIV-225***, *OIV-230*, *OIV-231*, ***OIV-232***, ***OIV-235***, ***OIV-236***, *OIV-241*
	Phenologhy	OIV-301, OIV-303
Quantitative	Yield variables	OIV-155, OIV-351, OIV-504
	Bunch, berry	OIV-202, **OIV-203**, **OIV-206**, OIV-502, OIV-503
	Must	OIV-233, OIV-505, OIV-506, OIV-508

Note: OIV descriptors in bold are those used for hierarchical clustering (dendrograms) and those in italics are those used forchecking the discrepancies between the descriptions considering the experience of the observer

©Vigne et Vin Publications Internationales (Bordeaux, France)

obtained for each of the descriptors. At least two repetitions of each description on each accessions were performed, selecting the modal value as a final description.

4. Molecular characterization: microsatellite analysis

DNA was extracted from young leaves using the DNeasy Plant kit (QIAGEN, Germany). A set of six microsatellite loci proposed by the GENRES 081 Project (European *Vitis* Database, www.genres.de/*vitis*/*vitis*. htm) was used in order to verify the varietal identity of each accession (table 1). Microsatellite analysis was performed as described by MARTIN *et al*. (2003), with one modifications (substitution for the ssrVrZAG47 microsatellite for VVMD27). PCR amplifications were analyzed by an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the fragments were sized with GeneMapper 4.0 software using GeneScan-LIZ 500 as internal marker (Applied Biosystems). To compare our results with another dataset different varieties were used as reference (Cabernet-Sauvignon, Chardonnay, Grenache (synonym Garnacha), Merlot and Mourvèdre; THIS *et al*., 2004).

5. Agronomic and must variables

The following agronomic variables were recorded: (1) yield-related variables like number of bunches per shoot, number of bunches per vine (at harvest), number of woody shoots per vine, yield (kilograms grapes per vine) and woody shoot weight (at pruning) and (2) bunch and berry-related variables like bunch length (stalk excluded), bunch width, stalk length, total bunch length, single bunch weight, berry weight and number of berries per bunch (recorded for 10 representative bunches).

The following must variables were recorded: (1) must yield (%) (OIV-233), following the methodology described by SANTIAGO *et al*. (2008): the supernatant obtained for this measurement was further used to determine (2) the probable alcohol content (%VOL) (OIV-505), which was first measured in ºBrix, using a hand held Brix refractometer (PR-101 Palette, ATAGO, Japan) and then converted to probable alcohol content (%VOL), using a conversion table; (3) pH (OIV-508) was measured using a pH meter (Crison Micro GLP21, Alella, Barcelona, Spain) and (4) total acidity (g/l tartaric acid; OIV-506) as described by SANTIAGO *et al*. (2008). For the preparation of must, berries from the central part of each representative bunch were selected and crushed.

6. Phenology

Phenology annotation, following the methodology of BAGGIOLINI (1952), was conducted three times a week throughout the growing season, except during flowering and veraison in which annotation was conducted daily.

7. Data analysis

The climate data were analyzed using descriptive statistics, whereas the agronomic and ampelographic description datasets were analyzed using both multivariate and univariate methods. Multivariate methods were used to observe the similarity between groups of varieties based on ampelographic data. Firstly, the ampelographic dataset was reduced by removing all unchanged characters. Hierarchical clustering was carried out using correlation coefficient (MARTÍNEZ DE TODA and SANCHA, 1997; CERVERA *et al*., 2001) and the « Unweighted Pair Group Method Analysis » (UPGMA) as linkage method (CERVERA *et al*., 2001; GARCÍA-MUÑOZ *et al*., 2005). For this analysis, 39 characters were selected (see table 2) and different dendrograms were generated for each study year.

Correspondence analysis (CA), based on the first ampelographic data matrix, was carried out to identify the most influential descriptors and their relationships with the described varieties (ALLEWELT and DETTWEILER, 1989; AGRESTI, 2002; GARCÍA-MUÑOZ *et al*., 2005). The correlations between 2006 and 2007 descriptions and 1998-1999 reference descriptions available in VGB were analyzed using a two-way Mantel test (LEGENDRE and LEGENDRE, 1998). This analysis has also been used to test if an isolated phenomenon such as a hailstorm generated differences on the descriptions.

Univariate methods comprised the Student´s t test for paired data to evaluate the existence of significant differences between the quantitative descriptors measured in 2006 and 2007 over the same individuals (CRAWLEY, 2007). Quantitative descriptors (i.e., quantitative characters according to OIV) were those of agronomic interest related to bunch, must and berry: berry weight, number of bunches per shoot, number of bunches per vine, yield (kg grapes per vine), number of woody shoots per vine, and weight of woody shoots per vine. Integer data variables (number of berries per bunch, number of bunches per woody shoot, number of bunches per vine, and number of vine shoots per vine) were transformed using log-transformation to normalise the dataset (CRAWLEY, 2007).

To test the existence of discrepancies between observers with respect to qualitative ampelographic descriptions, as well as the existence of different degrees of difficulty in describing the OIV descriptors, generalized linear models (GLM) were fitted with log-link function and Poisson errors (CRAWLEY, 2007). In this analysis, 35 characters were used (see table 2), excluding the quantitative parameters and the descriptors related to phenology. A contingency table was made relating the experience of observer and controls versus the number

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 3 - Ampelographic descriptions (bunch, berry, must) and agronomic parameters (yield variables) of the 27 accessions in 2006 and 2007.

	Variables	2006 [1]	2007 [1]	t-value	*p*-value	*df*
Yield variables	Number of bunches per shoot	1.10±0.09	0.65±0.04	4.942	<0.001	26
	Number of bunches per vine	8.00±0.62	6.31±0.38	2.284	0.016	25
	Number of woody shoots per vine	7.05±0.17	9.27±0.27	8.307	<0.001	26
	Yield (kg grapes per vine)	1.64±0.18	0.87±0.09	5.416	<0.001	26
	Woody shoot weight (g)	33.83±3.42	45.26±4.41	4.868	<0.001	26
Bunch, berry	Bunch length (stalk excluded) (cm)	16.84±0.58	17.81±0.55	1.702	0.1	26
	Bunch width (cm)	10.08±0.25	9.86±0.25	0.850	0.403	26
	Stalk length (cm)	4.45±0.22	3.23±0.16	5.501	<0.001	26
	Total bunch length (cm)	21.27±0.73	21.05±0.68	0.317	0.754	26
	Single bunch weight (g)	285.5±21.0	271.40±23.34	0.598	0.555	25
	Berry weight (g)	2.40±0.13	2.50±0.11	1.328	0.196	25
	Number of berries per bunch	165.9±15.4	137.2±10.3	2.397	0.012	25
Must	Must yield (%)	27.30±0.88	25.60±1.15	1.416	0.169	25
	Probable alcohol content (%VOL)	12.88±0.29	12.95±0.25	0.399	0.694	24
	pH	3.65±0.04	3.44±0.03	6.753	<0.001	24
	Total acidity (g/l TH_2)	4.72±0.21	5.66±0.20	3.839	<0.001	24

1 Data are the mean values (± standard error) of the 27 studied accessions

of occurrences presented at each level of expression for each one of the OIV descriptors. From these tables two models were fitted for each year (2006 and 2007), following the recommendations for measuring the correlation between observers proposed by AGRESTI (2002) and considering control observation as reference. The modal data of individual replicates for each descriptor was used as a control for the discrepancies, although in case of doubt the ampelographic photography description results were used. Finally, binomial tests for two proportions were used to analyze the differences between the presence/absence of teeth in the petiole sinus, number of wings per bunch and phenology for the years 2006 and 2007 (CRAWLEY, 2007). The proportions were calculated for each expression level of each OIV descriptor for each year.

The statistical analysis of similarity, hierarchical clusters, CA and Mantel test were performed with the Numerical Taxonomy System software (NTSYS v.2.1; ROHLF, 2000). The remaining analyses (generalized linear models, Student's t test for paired data and analysis for comparing two binomial proportions) were performed using R software environment (version 2.8, R DEVELOPMENT CORE TEAM, 2008).

RESULTS

1. Climatic data

The mean monthly temperature and precipitation observed during the 2006-2007 study period was similar to the 52-year averages, although small differences were detected (figure 1). In 2007, the rainfalls showed an atypical distribution. While the rainiest period of 2006 was winter (mid-September to mid-November), in 2007 the rainiest period was spring (March to June), mostly because of a hailstorm that took place on May 20th in which 43 mm were collected, representing 44% of that month's rain. This hailstorm phenomenon considering the geographic area studied occurs once every 5 years.

2. Differences among varieties using morphological and genetic approaches

The ampelographic description carried out in 2006 (supplementary table 1) showed a high degree of similarity between the repetitions of one single accession (figure 2). The lowest rate of similarity (0.85) was found in Batista variety due to a visible adaptation problem on the plot. Excluding this information, the similarity rate between repetitions of a single accession was very high, ranging from 0.92 (Pampolat girat, PAM.H24) to 0.99 (Manto Negro; MTN.E20). At a similarity level ≥ 0.75, four groups could be distinguished (figure 2). The first one, with a 0.75 similarity level among individuals, is formed by the red Beba Negra (BEN.E18) and Fogoneu accessions (FOG.E24). The second group, with a high similarity level among groups of 0.90, is formed by three red varieties, Excursach (EXC.E27), Callet (CAL.E26) and Manto Negro (MTN.E20; MTN.E29). These varieties generally have a very low-intensity colouration of the prostrate hairs of the tip and no or very few prostrate hairs between the main veins of both the young leaf and the

adult leaf. It must be stressed that the Callet and Manto Negro varieties had a 0.93 similarity coefficient. The third group, with a similarity between individuals of 0.81, is formed by a rosé variety (Beba roja, BER.E19) and three white varieties, Pensal blanco (PEN.SCL), Beba blanca (the similarity between the seven accessions studied is 0.91; BEB.E39; BEB.E14; BEB.E32; BEB.E43; BEB.O16; BEB.O17; BEB.O44) and Quigat (QUI.E33; QUI.E38). The last group, with a similarity between individuals of 0.78, is formed by the red varieties Eperó de gall (EPE.E37), Bobal (BOB.E11; BOB.E21), Gorgollassa (GOR.E25), Pampolat girat (PAM.H24), Giró (GIR.E31; GIR.E36), Mansés de Tibbus (MES.E30) and Sabaté (SAB.E35), and the similarity between the repetitions for the same accession was always higher than 0.90. In contrast with the other red varieties, these have an average or high number of hairs on the tip and also between the adult and young leaf's main veins. The Batista variety (BAT.E23) is separated from all the varieties, although it would be much closer to the last two groups, but with lower similarity level (0.61).

Regarding the descriptions of 2007 (supplementary data table 2), the rate of similarity between the repetitions of one single accession is very similar to the one observed in 2006 (figure 3). Again, the Batista variety (BAT.E23) showed the lowest similarity rate between repetitions (0.89). For the remaining accessions, the similarity rate for a single accession varies from 0.92 for Pensal blanco (PEN.SCL) and Pampolat girat (PAM.H24) to 0.98 for Callet (CAL.E26), Beba roja (BER.E19) and Sabaté (SAB.E35). The grouping of varieties in 2007 largely agrees with the grouping obtained with the 2006 data, although with certain differences that could be due to the damage caused by the hailstorm.

The Mantel test showed a good correlation among all the descriptions. The 2006 and 2007 descriptions (updated descriptors) showed a correlation rate of 0.87 and 0.74 with the 2006 and 1998-1999 (reference descriptions) respectively. The best correlations between 2006 and 2007 were obtained in shoot tip (r=0.92), and the worst correlation was obtained in vine shoot (r=0.61), whereas mature leaf (r=0.75), berry (r=0.69) and bunch (r=0.65) showed intermediate correlation coefficients.

Table 4 - Qualitative ampelographic characters which showed significant differences between the ampelographers and control descriptions in 2006 and 2007 years.

	2006	2007
Descriptor	*p* (Chi) [1]	*p* (Chi) [1]
OIV-002	ns	<0.001
OIV-003	<0.001	ns
OIV-008	<0.001	ns
OIV-051	<0.001	0.002
OIV-067	0.001	ns
OIV-068	<0.001	ns
OIV-075	<0.001	ns
OIV-080	<0.001	ns
OIV-209	0.02	ns
OIV-232	<0.010	0.002
OIV-235	<0.010	<0.001

[1]: No significant differences (ns)

Based on the genetic profiles obtained at six SSR loci the 27 accessions were grouped into 16 different genotypes (table 1). The white accessions E39, O16, O17, O44, E14, E32 and E43 showed the same microsatellite profile as the Beba roja rosé variety (E19). The accessions pairs "E11 and E21", "E31 and E36", "E20-E29" and "E33-E38" also showed the same microsatellite profiles. These results are in agreement with the ampelographic descriptions.

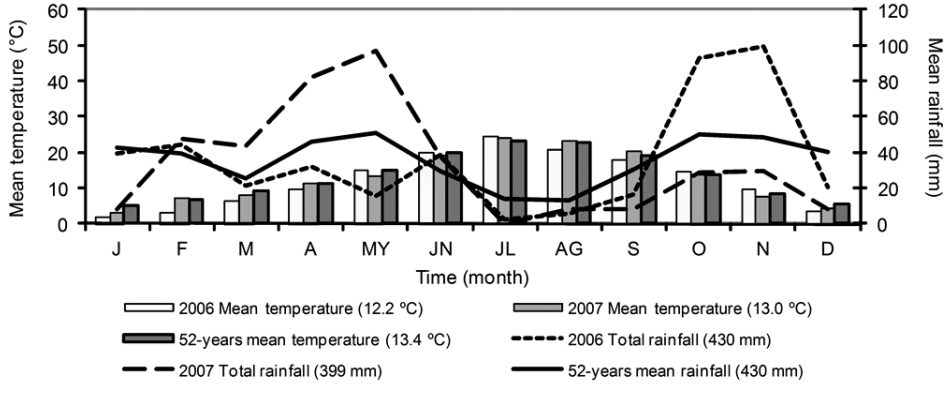


Figure 1 - Schematic distribution of mean monthly temperature and rainfall for the study period (2006 and 2007). The 52-year mean monthly data were used as reference.
Data based on "El Encín" Meteorological Station (40º31´N, 3º17´W, 610 m asl, Alcalá de Henares, Madrid, Spain)

©Vigne et Vin Publications Internationales (Bordeaux, France)

3. The most influential descriptors

The CA results are shown separately for each of the years studied (figure 4). The OIV-001, OIV-016, OIV-230, OIV-231 and OIV-241 descriptors were stable in 2006 and 2007, as well as the OIV-081-2 descriptor in 2007. The first two axes of the CA in the description of the year 2006 explained 52% of the variance (figure 4a). The first axis absorbed 30% of the variance, with OIV-225 and OIV-087 being the most distinguishing characteristics. The second axis explained 22% of the variance and was related with the OIV-084, OIV-053 and OIV-004 descriptors. The CA of 2007 showed that variance explained by the first two axes was 49% (figure 4b). Axis 1 explained 28% of the variance and was related with the OIV-225, OIV-084, OIV-053 and OIV-004 descriptors. Axis 2 explained 21% of the variance and it was mostly influenced by the OIV-202 and OIV-070 descriptors. These results were largely in line with the grouping of varieties in the 2006 and 2007 dendrograms.

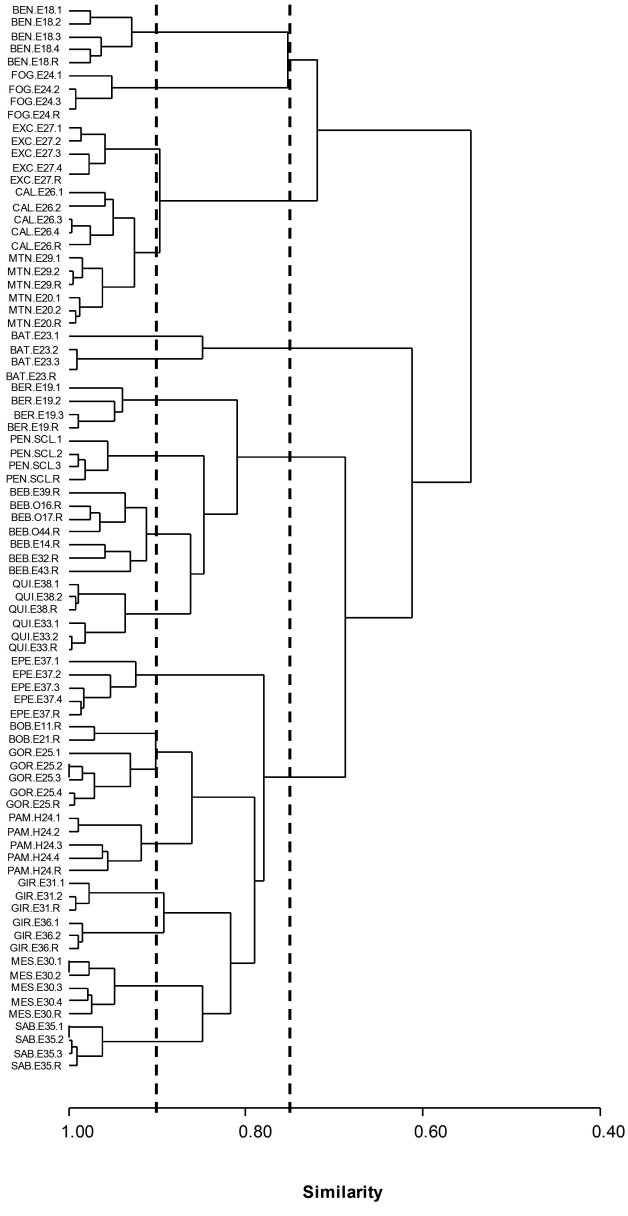


Figure 2 - Similarity among the repetitions of the 27 accessions bases on the 2006 ampelographic descriptions and revealed by Cluster dendrogram, using UPGMA method and correlation coefficient.

Codes in Tab 1. The number after accessions indicates repetitions and R is the modal result of the descriptions realized. Lines indicate 0.75 and 0.90 similarity cuts

4. Differences in ampelographic descriptions, agronomic and phenological characters

The differences between the 2006 and 2007 ampelographic quantitative datasets were statistically significant (z-value=2.461, p-value=0.014) for the presence of teeth in the petiole sinus of adult leaf, being more frequent in 2006 that in 2007 (63% compared to 30%). With respect to the number of bunch wings, there were also statistically significant differences. In 2006, 7% of bunches had no wings, while in 2007, there was no bunch without wings (z-value=2.836, p-value=0.004). In addition, in 2007 there was a larger number of bunches with 1 or 2 wings (93%) in contrast to 2006 (67%; z-value=2.388, p-value=0.016).

The agronomic parameters showed statistically significant differences (table 3). The number of bunches per shoot in 2006 was almost double that of 2007 (1.10 compared to 0.65), the vines had an average number of 8 bunches in 2006, while in 2007 this number was reduced to 6.31. Grape production (yield) was greater in 2006 (1.64 kg per vine) than in 2007 (0.87 kg grapes per vine). The number of woody shoots per vine and the weight of vine shoot also showed statistically significant differences, however, in contrast to the parameters cited above, they were greater in 2007 (from 7 to 9 woody shoots per vine and from 34 to 45 g per woody shoot) compared to 2006. Of the seven quantitative parameters related to bunch and berry, two showed significant differences: the stalk length and the number of berries per bunch. In both cases, the values were higher for 2006 than for 2007 (4.45 cm vs. 3.23 cm and 166 vs. 137, respectively) (table 3).

The parameters related to the characterization of must (table 3) showed significant changes in pH and total acidity, the 2007 samples were more acidic and thus had a lower pH than the 2006 samples.

Regarding to duration of the vegetative period, all the plants were at the same phenological stage when the hailstorm occurred: flower buds separated (stage H; data do not shown). Until that moment the duration of the vegetative period was the same for the two studied years (28 days from stage C (budbreak) to stage H (flower buds

©Vigne et Vin Publications Internationales (Bordeaux, France)

separated)), and therefore no significant differences were found in this period between 2006 and 2007. However, the differences were marked from that moment until ripeness, since from stage H to stage N (ripeness) all the cultivars showed longer vegetative periods in 2007 (t-values>1.706, p-values <0.05). Overall, there were significant differences between the two studied years (t-value=4.491, p-value <0.001), being the duration of the vegetative period which was longer in 2007 (144 days) than in 2006 (136 days).

5. Ampelographer's experience and objectivity influenced qualitative ampelographic descriptions

For the year 2006, there was not independence between the ampelographer and the description for 10 out of the 35 characters studied (28.6% of the total; table 4), suggesting that observer's experience had an influence over the description of these characters. The 10 characters were the anthocyanin colouration of the prostrate hairs of the tip (OIV-003), the colour of the ventral side of the internodes (OIV-008), the colour of the upper side of the fourth leaf (OIV-051), the shape of the blade (OIV-067), the number of lobes (OIV-068), the blistering of the upper side (OIV-075), the shape of the base of the petiole sinus (OIV-080), the number of the bunch wings (OIV-209), the juiciness of the flesh (OIV-232) and the firmness of the flesh (OIV-235). For the year 2007, the observer's experience only had an influence over the descriptions of 4 characters (11.4% of the total; table 4), the anthocyanin colouration of the shoot tip (OIV-002), the colour of the young leaf's upper side (OIV-051), the juiciness of the flesh (OIV-232) and the firmness of the flesh (OIV235).

DISCUSSION

Ampelographic descriptions and molecular analyses have proved to be very useful tools when describing *Vitis vinifera* accessions from the Balearic Islands. Ampelography has been used in this study as a preliminary technique for the clarification of plant material, and the results were later confirmed by microsatellite analysis. Although the varieties showed a high phenotypic similarity, we found a few key characteristics (OIV-225, OIV-084, OIV-053 and OIV-004) for their identification (TRUEL and BOURSIQUOT, 1986). Moreover, it has been seen that ampelographic descriptions are influenced by occasional climatic phenomena (hailstorm) (DETTWEILER, 1993), which mainly affect vine shoots, bunches, agronomic characteristics, and berry composition (CALÒ *et al.*, 1996). The experience of the ampelographers also has an influence on the ampelographic description. In fact, the subjectivity of certain characteristics, in addition to thecampelographer's lack of experience, is a factor that complicates the correct description of plant material (ORTIZ *et al.*, 2004).

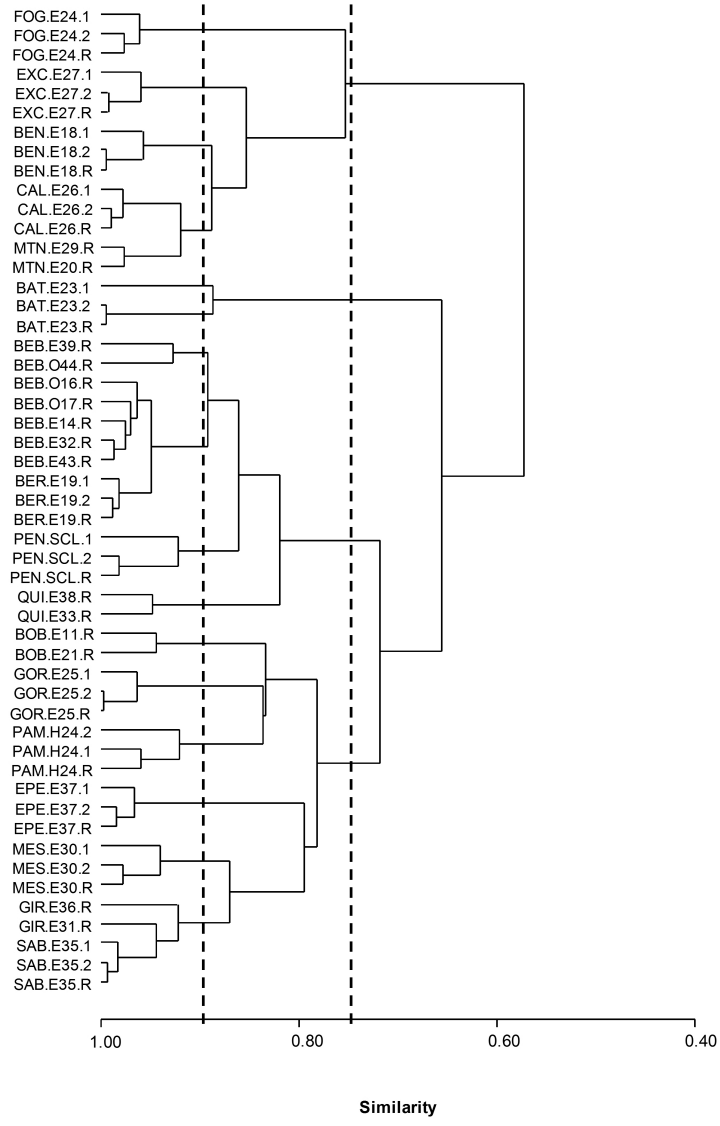


Figure 3 - Similarity among the repetitions of the 27 accessions bases on the 2007 ampelographic descriptions and revealed by Cluster dendrogram, using UPGMA method and correlation coefficient.

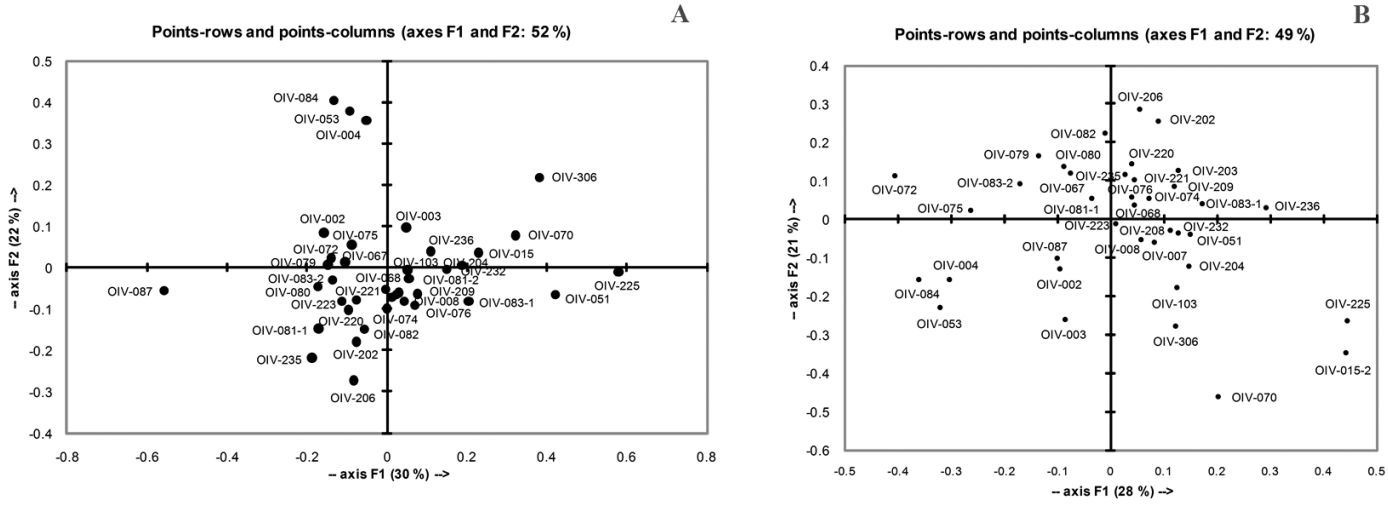
Codes in table 1. The number after accessions indicates repetitions and R is the modal result of the descriptions realized. Lines indicate 0.75 and 0.90 similarity cuts.

1. Differences among varieties using morphological and genetic analysis

The morphological grouping of the varieties for which several accessions were studied (Beba blanca, Bobal, Giró, Manto Negro and Quigat) showing similarity rates of over 0.90 in the two studied years. This is the minimum rate for verifying homonyms and for clone selection processes (CERVERA *et al.*, 2002). The slight differences could be related with the typical variability of the plant material (BESSIS, 2007), the polyclonal origin of the vine populations (KOZJAK *et al.*, 2003), changes in the virus load, epigenetic differences, somatic mutations or several

©Vigne et Vin Publications Internationales (Bordeaux, France)

Figure 4 - Source of variation for the OIV descriptors as projected by correspondence analysis (CA). (a) descriptions of 2006, (b) descriptions of 2007 data



combinations of these effects (GONZÁLEZ-TECHERA *et al*., 2004). The genetic analyses confirm this hypothesis, since these accessions showed the same at six SSR loci. On the opposite, Callet and Manto Negro varieties were grouped at a similarity level of 0.93 in the two years of the study, however, they belong to different varieties according to the microsatellite analysis. Similar cases have been described in literature and they point towards a parentage relationship between them (HINRICHSEN *et al*., 2001). This hypothesis has been probed, since due to their particular geographic location (both are only found in the Balearic Islands), these varieties are the result of natural cross sharing the same mother parent (GARCÍA-MUÑOZ *et al*., 2011). A similar situation has been described in the Canary Islands (Spain) for Listan Negro which originated from the crossing of two indigenous varieties, Negramoll and Listán blanco (ZEROLO and CABELLO, 2006).

The case of Beba blanca and Beba roja varieties is remarkable. They had similarity levels of 0.81 and 0.90 in the morphological analyses in 2006 and 2007, respectively, but showed identical SSR profiles. According to MARTÍNEZ DE TODA and SANCHA (1997), varieties with similarity rates below 0.85 could be considered as different varieties. Since these two varieties have identical genotypes but clear morphological differences with respect to berry colour (one is a white and the other is a rosé variety) that could be explained by the incidence of somatic mutations and therefore they should be considered as different cultivars (LAIADI *et al*., 2009).

2. The most influential descriptors

The descriptors that must be included for ampelographic description on this type of plant material are the colour of the berry (OIV-225), since white, red and rosé varieties were studied, the density of prostrate hairs between the main veins of the adult leaf's lower side (OIV-084) (MARTÍNEZ DE TODA and SANCHA, 1997) and the density of hairs between the main veins of the young leaf (OIV-053) (ALLEWEDT and DETWEILER, 1989; MARTÍNEZ DE TODA and SANCHA, 1997). These were the most discriminating characteristics and had the stronger influence on the grouping of the varieties studied.

3. Differences in ampelographic descriptions, agronomic and phenological characters

The hailstorm phenomenon seems to be the cause of the great influence of "year" on the agronomic characterization parameters, since it increased rainfall during the flowering period. By contrast, temperature was largely in line between 2006 and 2007 data. The plant material was greatly affected by the hailstorm as 90% of the grapevines were damaged. The most affected vine parts were the young shoot and the bunch, whereas the least affected part turned out to be the shoot tip.

The differences observed in leaves between 2006 and 2007 were mainly due to the presence of teeth in the petiole sinus. This characteristics has proved to be stable and objective (ORTIZ *et al*., 2004) and, according to the results of this work, was easily identifiable by ampelographers. Therefore, the statistically significant differences found between the two years of study could be blamed on this

©Vigne et Vin Publications Internationales (Bordeaux, France)

occasional climatic phenomenon. Since the hailstorm strongly reduced the length of the young shoots, and more specifically when the material to be described were selected, this came from well-developed side shoots. It seems that this characteristic was found more in adult leaves of the centre part of the shoots than in well-developed side shoots. In cases of meteorological accidents, special attention must be paid when making the descriptions of young vine branches and bunches, because this material is the most damaged.

The hailstorm has also had an impact on change some bunch-related such as the number of bunch wings, increasing the number of bunches with 2 wings and lowering the number of bunches with 3 wings in 2007. This may have been due to either (a) the hailstorm by damaging certain parts of the bunch increased the exposure of the wings, or (b) the bunch in its initial stage is capable to develop secondary growths as observed in vine shoots (PRATT, 1971). Unfortunately we do not have evidence of the real causes. The number of grapes per bunch was significantly less in 2007 than in 2006. The total length of the bunch was similar in 2006 and 2007, however, the length of the stalk varied a great deal. This has been shown by THEILER and COOMBE (1985) where in occasional losses of flowers in the flower head, the growth of the bunch stalk stops immediately.

While the ampelographic characteristics of each variety remained more or less constant during the studied period, the climatic conditions of each year had an influence on the agronomic characteristics (SANTIAGO *et al*., 2008), and more specifically on yield. As a result of the hailstorm there was a loss of a large number of flower heads like was described by REYNIER (2005), which was caused by both the reduction in the number of bunches per vine and the number of berries per bunch. This phenomenon also caused a drastic yield drop (kg grapes per vine) during 2007. A similar case was described by JONES and DAVIS (2000) where rainfall during physiologically important periods (flowering and maturation) tended to decrease crop production. However, no significant differences were found in berry weight, contrasting with the findings of JONES and DAVIS (2000). With the vine branches being significantly damaged, an increase in secondary ramifications was caused and more suckers appeared on the shoots (REYNIER, 2005), which caused the weight of pruning wood, the number of vine shoots per vine and the weight of the vine shoots to increase, this being a sign of energy related to grape production explained by RABINO *et al*. (2000).

The hailstorm also caused a delay in phenology that affected the vegetation by prolonging its vegetative period and affecting the quality of the harvest due to the heterogeneity of the condition of bunches as showed by REYNIER (2005). With regards to must parameters, the only value that showed no differences between the years of study was the probable alcohol content that was more related to stability than to changes in environmental conditions (JONES and DAVIS, 2000). In this study, both acidity and pH were extremely influenced by the environment, similar results were found by PRENESTI *et al*. (2004). As pointed out by JONES and DAVIS (2000), years characterized by a delay in phenology are years in which the levels of acidity increased. The same occurred for the pH data, since these two variables are negatively related (ZAMBONI *et al*., 1997).

4. Ampelographer's experience and objectivity influenced qualitative ampelographic descriptions

The most discriminating characteristics do not change with the environment (DETTWEILER, 1993). They are also stable and objective (ORTIZ *et al*., 2004) and in this study, also these descriptors showing no differences between ampelographers. Certain characteristics were more easily assessable than others, because in neither of the two years of study were found differences among ampelographers when compared with the control. With regard to the discriminating characteristics that showed differences among the ampelographers, it should be pointed out that in the second year of the study the number of descriptors that were evaluated differently dropped considerably. This result supports the idea of that the ampelographers's experience is very important ORTIZ *et al*. (2004). In any case, the characteristics that define the juiciness of the berry flesh (OIV-232) and the firmness of the berry flesh (OIV-235) have proved to be variable in the two years of study and could be considered subjective. Both of these characteristics can also vary with the period in which the descriptions are made due to the change in berry compositions throughout ripening (GIOVANELLI and BRENNA, 2007). Furthermore, even though the colour of the upper side of the fourth leaf (OIV-051) characteristic is considered by the OIV (1984) as easy to assess, in this work it was not considered as such, and the significant differences were found compared with the control in the two studied years.

CONCLUSIONS

The combination of morphological and molecular characterizations allowed to group the 27 accessions collected in the Balearic Islands (Spain) into 17 different grapevine cultivars. The accurate selection of the plant material to be evaluated is very important for developing a correct ampelographic description since it could influence some characteristics, especially the presence of teeth on the adult leaf and the number of the bunch wings. The "colour of the upper side of the fourth leaf" descriptor

©Vigne et Vin Publications Internationales (Bordeaux, France)

(OIV-051) should be excluded from the list of easy-to-assess characteristics issued by the OIV, since it showed significant differences with the control in both years of study.

The hailstorm influenced agronomic characterization: decrease bunch weight, number of berries per bunch, and yield (kg grape per vine) stand out. This meteorological accident also had a high influence on must quality by increasing acidity and decreasing pH. In addition, the hailstorm influenced the phenology by increasing the duration of the vegetative period.

Ampelographers´ experience is another factor that could affect the descriptions, but this work showed that a training process exists. Considernig the two consecutive years of study, the number of characteristics for which the ampelographers' description varied from the reference description dropped considerably in the second year.

The morphological evaluations point towards a strong genetic relationship among the studied accessions. This hypothesis is supported by the common origin of all varieties, since all varieties are from the Balearic Islands (Spain). In order to corroborate the possible genetic relationships, further analyses involving a larger set of molecular markers are required. Finally, the genetic analysis shows Beba blanca as a possible somatic mutant from Beba roja.

Acknowledgements: The authors would like to thank Thierry Lacombe for critical reading of the manuscript and Josu G. Alday for statistical advice. Financial support from INIA (National Institute for Agricultural and Food Research, Spain), project RTA 04 175-C3-3 and ERDF (European Regional Devolpment Fund).

REFERENCES

AGRESTI A., 2002. *Categorical Data Analysis.* John Wiley & Sons, New York.

ALLEWELDT G. and DETTWEILER E., 1989. A model to differentiate grapevine cultivars with the aid of morphological characteristics. *Riv. Vitic. Enol.*, **42**, 1, 59-63.

BAGGIOLINI M., 1952. Les stades repères de la vigne. *Rev. Suisse Vitic. Arboric. Hortic.*, **25**, 1, 7-9.

BESSIS R., 2007. Evolution of the grapevine (*Vitis vinifera* L.) imprinted by natural and human factors. *Can. J. Bot.*, **85**, 8, 679-690.

BOWERS J.E., BANDMAN E.B. and MEREDITH C.P., 1993. DNA fingerprint characterization of some wine grape cultivars. *Am. J. Enol. Vitic.*, **44**, 3, 266-274.

CABEZAS J.A., CERVERA M.T., ARROYO-GARCÍA R., IBÁÑEZ J., RODRÍGUEZ-TORRES I., BORREGO J., CABELLO F. and MARTÍNEZ-ZAPATER J.M., 2003. Garnacha and Garnacha Tintorera: Genetic relationships and the origin of teinturier varieties cultivated in Spain. *Am. J. Enol. Vitic.*, **54**, 4, 237-245.

CALÒ A., TOMASI D., CRESPAN M. and COSTACURTA A., 1996. Relationship between environmental factors and the dynamics of growth and composition of the grapevine. *Acta Hortic.*, **427**, 217-231.

CERVERA M.T., CABEZAS J.A., RODRÍGUEZ-TORRES I., CHÁVEZ J., CABELLO F. and MARTÍNEZ-ZAPATER J.M., 2002. Varietal diversity within grapevine accessions of cv. Tempranillo. *Vitis*, **41**, 1, 33-36.

CERVERA M.T., RODRÍGUEZ I., CABEZAS J.A., CHÁVEZ J., MARTÍNEZ-ZAPATER J.M. and CABELLO F., 2001. Morphological and molecular characterization of grapevine accessions known as Albillo. *Am. J. Enol. Vitic.*, **52**, 2, 127-135.

CHOMÉ P., SOTÉS V., BENAYAS F., CAYUELA M., HERNÁNDEZ M., CABELLO F., ORTIZ J., RODRÍGUEZ-TORRES I. and CHAVES J., 2003. *Variedades de vid. Registro de variedades comerciales.* MAPA, Madrid.

CRAWLEY M.J., 2007. *The R book*. John Wiley & Sons, Chichester.

CRESPAN M., CALÒ A., GIANNETTO S., SPARACIO A., STORCHI P. and COSTACURTA A., 2008. Sangiovese and Garganega are two key varieties of the Italian grapevine assortment evolution. *Vitis*, **47**, 2, 97-104.

CUNHA J., TEIXEIRA SANTOS M., CARNEIRO L.C., FEVEREIRO P. and EIRAS-DIAS J.E., 2009. Portuguese traditional grapevine cultivars and wild vines (*Vitis vinifera* L.) share morphological and genetic traits. *Genet. Resour. Crop. Evol.*, **56**, 7, 975-989.

DE MATTIA F., IMAZIO S., GRASSI F., LOVICU G., TARDAGUILA J., FAILLA O., MAITT C., SCIENZA A. and LABRA M., 2007. Genetic characterization of Sadinia grapevine cultivars by SSR markers analysis. *J. Int. Sci. Vigne Vin*, **41**, 4, 175-184.

DETTWEILER E., 1993. Evaluation of breeding characteristics in *Vitis*. Influence of climate on morphologic characteristics of grapevines. *Vitis*, **32**, 4, 249-253.

GARCÍA-MUÑOZ S., LACOMBE T., DE ANDRÉS M.T., GAFORIO L., MUÑOZ-ORGANERO G., LAUCOU V., THIS P. and CABELLO F., 2011. Grape varieties (*Vitis vinifera* L.) from the Balearic Islands: genetic characterization and relationship with Iberian Peninsula and Mediterranean Basin. *Genet. Resour. Crop. Evol.* doi: 10.1007/s10722-011-9706-5.

GARCÍA-MUÑOZ S., MARTÍN A., YUSTE J., ARRANZ C. and RUBIO J.A., 2005. Caracterización ampelográfica de la variedad tinta de vid Rufete (*Vitis vinifera* L.) en viñedos de la Sierra de Francia (Salamanca). *In: 5º Congreso SECH. Asociación Portugesa de Ciencias Hortícolas.* Ed. Oporto, Vol II, p. 376-383.

GIOVANELLI G. and BRENNA O.V., 2007. Evolution of some phenolic components, carotenoids and chlorophylls during ripening of three Italian grape varieties. *Eur. Food Res. Technol.*, **225**, 1, 145-150.

©Vigne et Vin Publications Internationales (Bordeaux, France)

GONZÁLEZ-TECHERA A., JUBANY S., PONCE DE LEÓN I., BOIDO E., DELLACASSA E., CARRAU F.M., HINRICHSEN P. and GAGGERO C., 2004. Molecular diversity within clones of cv. Tannat (*Vitis vinifera* L.). *Vitis*, **43**, 4, 179-185.

HINRICHSEN P., NARVÁEZ C., BOWERS J.E., BOURSIQUOT J.M., VALENZUELA J., MUÑOZ C. and MEREDITH C.P., 2001. Distinguishing Carmenère from similar cultivars by DNA typing. *Am. J. Enol. Vitic.*, **52**, 4, 396-399.

JONES G.V. and DAVIS R.E., 2000. Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. *Am. J. Enol. Vitic.*, **51**, 3, 249-261.

KOZJAK P., KOVOSEK-KORUZA Z. and JAVORNIK B., 2003. Characterisation of cv. Refosk (*Vitis vinifera* L.) by SSR markers. *Vitis*, **42**, 2, 83-86.

LAIADI Z., BENTCHIKOU M.M., BRAVO G., CABELLO F. and MARTINEZ-ZAPATER J.M., 2009. Molecular identification and genetic relationships of Algerian grapevine cultivars maintained at the germplasm collection of Skikda (Algeria). *Vitis*, **48**, 1, 25-32.

LEGENDRE P. and LEGENDRE L., 1998. *Numerical ecology*. Elsevier Science BV, Amsterdam.

MARÉS H., 1890-1891. *Description des cépages de la Région Méditerranée de la France*. Coulet, Montpellier.

MARTÍN J.P., BORREGO J., CABELLO F. and ORTIZ J.M., 2003. Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers. *Genome*, **46**, 1, 10-18.

MARTÍNEZ DE TODA F. and SANCHA J.C., 1997. Ampelographical characterization of red *Vitis vinifera* L. cultivars preserved in Rioja. *Bull. OIV*, **70**, 793-794, 220-234.

OIV (Office International de la Vigne et du Vin), 1984. *Codes des caractères descriptifs des variétés et espèces de Vitis*. Ed. Dedon, Paris.

ORTIZ J.M., MARTÍN J.P., BORREGO J., CHÁVEZ J., RODRÍGUEZ I., MUÑOZ G. and CABELLO F., 2004. Molecular and morphological characterization of a *Vitis* gene bank for the establishment of a base collection. *Genet. Resour. Crop. Evol.*, **51**, 4, 403-409.

PAVEK D.S., LAMBOY W.F. and GARVEY E.J., 2003. Selecting in situ conservation sites for grape genetic resources in the USA. *Genet. Resour. Crop. Evol.*, **50**, 2, 165-173.

PRATT C., 1971. Reproductive anatomy in cultivated grapes. A review. *Am. J. Enol. Vitic.*, **22**, 2, 92-109.

PRENESTI E., DANIELE P.G., TOSO S., ZELANO V. and BERTO S., 2004. Development of a speciation model for the interpretation of the acid-base properties of grape red wines. *Chem. Spec. Bioavail.*, **16**, 1-2, 17-24.

R DEVELOPMENT CORE TEAM, 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.

RABINO M., BONIFACINO G., TRANGNI R. and REGIO R., 2000. Considerazione vitienologiche su selezioni clonali di Cortese. *Vignevini*, **27**, 5, 42-46.

REYNIER A., 2005. *Manual de viticultura*. Mundi Prensa, Madrid.

ROHLF F.J., 2000. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software: Setauket, New York.

SANTIAGO J.L., BOSO S., GAGO P., ALONSO-VILLAVERDE V. and MARTÍNEZ M.C., 2008. A contribution to the maintenance of grapevine diversity: The rescue of Tinta Castañal (*Vitis vinifera* L.), a variety on the edge of extinction. *Sci. Hortic.*, **116**, 2, 199-204.

SANTIAGO J.L., BOSO S., VILANOVA M. and MARTÍNEZ M.C., 2005. Characterisation of cv. Albarín Blanco (*Vitis vinifera* L.). Synonyms, homonyms and errors of identification associated with this cultivar. *J. Int. Sci. Vigne Vin*, **39**, 2, 57-65.

SCHNEIDER A., TORELLO MARINONI D. and CRESPAN M., 2008. Genetics and ampelography trace the origin of Muscat fleur d'oranger. *Am. J. Enol. Vitic.*, **59**, 2, 200-204.

THEILER R. and COOMBE B.G., 1985. Influence of berry growth and growth regulators on the development of grape peduncles in *Vitis vinifera* L. *Vitis*, **24**, 1-11.

THIS P., JUNG A., BOCCACCI P., BORREGO J., BOTTA R., COSTANTINI L., CRESPAN M., DANGL G.S., EISENHELD C., FERREIRA-MONTEIRO F., GRANDO M.S., IBÁÑEZ J., LACOMBE T., LAUCOU V., MAGALHÃES R., MEREDITH C.P., MILANI N., PETERLUNGER E., REGNER F., ZULINI L. and MAUL E., 2004. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theor. Appl. Genet.*, **109**, 7, 1448-1458.

TRUEL P. and BOURSIQUOT J.M., 1986. Etudes sur le material introduit dans les collections ampélographiques en vue de son identification et de la recherche de synonymes. *Vignevini*, **13** (suppl. al nr. 12), 81-85.

UPOV (International Union for the Protection of New Varieties of Plants), 2007. TG 50/9, 2007/06/14.

ZAMBONI M., IACONO F. and BOZZALLA L., 1997. Influenze ambientali sulle caratteristiche produttive e qualitative dei vitigni Biancolella e Forastera nell'isola d'Ischia. *Vignevini*, **24**, 4, 9-15.

ZEROLO J. and CABELLO F., 2006. *Variedades de vid de cultivo en Canarias*. Instituto Canario de Calidad Agroalimentaria, Santa Cruz de Tenerife.

©Vigne et Vin Publications Internationales (Bordeaux, France)