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TESIS DOCTORAL:

ESTUDIO DE VARIEDADES MINORITARIAS DE VID (*Vitis vinifera* L.): DESCRIPCIÓN, CARACTERIZACIÓN AGRONÓMICA Y ENOLÓGICA DE MATERIAL PROCEDENTE DE LAS ISLAS BALEARES

STUDY OF MINOR GRAPEVINE CULTIVARS (*Vitis vinifera* L.): DESCRIPTION, AGRONOMIC AND OENOLOGICAL CHARACTERIZATION OF VARIETIES FROM THE BALEARIC ISLANDS

Presentada por Sonia García Muñoz para optar al grado de doctora por la Universidad de Valladolid

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(Montero R. 2008. *Instrucciones para salvar el mundo*. Madrid, Spain: Alfaguara)

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Abstract

The global oenological market is driving a cultivated grapevine homogenization around the word. The loss of cultivars (*Vitis vinifera* L.) is an important problem in most of the viticulture areas that must be stopped to conserve the varietal heritage.

The oenological market is changing, since nowadays the wine consumers are demanding new wines styles, based on the originality, quality, and link to the “terroir” and with historical background. Therefore it is necessary to satisfy these new requirements. The minor varieties, most of them under risk of extinction, could be a great option to satisfy this demand, since these varieties are perfectly adapted to the local environmental conditions. However the oenological potential of most of these cultivars is unknown.

In this thesis, it has been studied a group of minor grapevine cultivars collected from 1914 to 2000 mainly in the Balearic Islands (Spain) and nowadays preserved in the *Vitis* Germplasm Bank VGB “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain). A preliminary step to offer new grapevine material to vinegrowers and winemakers is to identify perfectly the cultivars, with that purpose ampelographic and microsatellite analysis were used. The historical links between the cultivars and the Balearics Islands have been analyzed. It is also essential for promoting a new cultivar to know the agronomical behaviour, including resistance to disease as powdery mildew (*Erysiphe necator* Schwein.), and the oenological potential, based on aromatic grape characterization and wine sensorial analysis. All of these parameters have been analysed in this study.

In general, the studied cultivars showed great agronomical behaviour and most of them also showed a high resistance to powdery mildew. The genetic analysis has pointed out that two gene pools exist. The oenological potential of the minor varieties has been proved, since most of them were well accepted by wine experts in sensorial analysis. The knowledge of the oenological potential of the minor varieties could be one of the last opportunities that minor varieties have to survive in the future.

Resumen

La globalización del mercado enológico está conduciendo a una homogeneización de las variedades de vid cultivadas alrededor del mundo. La pérdida de variedades (*Vitis vinifera* L.) es un problema importante en la mayoría de las áreas vitícolas, este hecho debe detenerse para conservar el patrimonio varietal.

El mercado enológico está en constante movimiento, actualmente los consumidores están demandando nuevos estilos de vino basados en la originalidad y calidad, otra tendencia es el consumo de productos de proximidad, ligados al territorio, que representen los antecedentes históricos del mismo. Por lo tanto, es necesario satisfacer estos nuevos requerimientos. Las variedades minoritarias, la mayoría en peligro de extinción, pueden ser una buena opción para satisfacer esta nueva demanda, ya que estas variedades se encuentran perfectamente adaptadas a las condiciones locales y medioambientales. Sin embargo, el potencial enológico de estas variedades sigue siendo desconocido.

En esta tesis se ha estudiado un grupo de variedades minoritarias de vid recogidas entre los años 1914 y 2000 principalmente en las Islas Baleares (España) y conservadas en la actualidad en el Banco de Germoplasma de Vid BGV “Finca El Encín” (IMIDRA, Alcalá de Henares, Madrid, España). Un paso preliminar para poder ofrecer a los viticultores y enólogos nuevo material vitícola es identificar perfectamente a las variedades, para ello se han realizado descripciones ampelográficas y análisis genéticos basados en el uso de marcadores moleculares. A su vez, se ha analizado la relación histórica entre las variedades y las Islas Baleares. Otro aspecto fundamental a la hora de promover nuevas variedades de vid es conocer el comportamiento agronómico, incluyendo la resistencia a enfermedades como el oídio (*Erysiphe necator* Schwein.), y el potencial enológico, basado en la caracterización aromática de las uvas y el análisis sensorial de los vinos realizados. Todos estos parámetros se han analizado en este trabajo.

En general, las variedades estudiadas mostraron un buen comportamiento agronómico. Además la gran mayoría mostró una alta resistencia al oídio. El análisis genético ha indicado la existencia de dos reservas genéticas. Las variedades minoritarias han mostrado un alto potencial enológico, al ser aceptados la mayoría de sus vinos por los expertos en el análisis sensorial. El conocimiento del potencial enológico de las variedades minoritarias puede ser una de las últimas oportunidades que tienen estas variedades para sobrevivir en el futuro.

Chapter 1

General view of *Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain)

Introduction

Introduction

"I am writing this booklet to spread the most important knowledge about the plague that destroys all the vineyard in Europe and to do that these knowledge arrive to the heart of the viticulture villages in this province, for all understands the grave danger of which are threatened and you know the reserved place in the great battle for self-defense that, with no doubt, we will have to fight. The agriculture, the industry and the trade are founds of richness of this province. Death of the grapevine is the collapse of the viticultural country, it is not only the decadence, it is an obstacle for the prosperity along a very extend period of time" (Pou y Bonet 1880). These words were written to avoid the entry of the phylloxera in the Balearic Islands (Spain). Unfortunately, it was not enough because the phylloxera plague appears in 1891 destroying much of the vineyard in the Balearic Islands (Ballester 1911). However, more than a century later, the lost of the grapevine growing area and grapevine diversity is a current problem in most of the viticulture areas in the world including the Balearic Islands.

Based on DNA profiling results, there are around 5,000 grapevine varieties (*Vitis vinifera* L.; This et al. 2006), however evidences of grapevine diversity loosing have been appointed (Bessis 2007; Carimi et al. 2010). Actually, international varieties, as Cabernet-Sauvignon, Sauvignon blanc or Chardonnay, are present in most of the European countries as a consequence of wine globalization market. Hence a few varieties are increasing the vineyard area worldwide, reducing drastically the local cultivars and consequently the gene pool. Nowadays, the Spanish viticulture area is divided on 73 Designations of Origin (DO) and includes 250 different cultivars in its national grapevine catalogue, being the fourth country in grapevine diversity in the European Union behind Italy, Portugal and France (Lacombe et al. 2011). Despite of the high number of autochthonous cultivars the international varieties are widely spread and their cultivation are allowed in most of the Spanish DO. In contrast, most of the minor varieties that could be called "autochthonous" are not included in any Designations of Origin (Cabello 2004). In fact, the 95% of the Spanish vineyard area is cultivated with only 34 cultivars; as a consequence, the local cultivars have decreased over the years especially in their natural environmental conditions (This et al. 2006; Cipriani et al. 2010). These problems are also present in the Balearic Islands where the loss of cultivars remains unknown.

The introduction of grapevine foreign material induces important changes in the local viticulture, especially in isolated areas which are most sensitive to changes. In fact, some of the ancient cultivars sited in the Balearic Islands are not found in natural conditions nowadays, fortunately they are conserved in grapegene repositories. Actually, only four varieties (Callet, Manto Negro, Pensal Blanca and Fogoneu) out of 20 allowed in the two out of the 73 Spanish Designations of Origin located in the Balearic Islands are local varieties, the rest are internationally spread as

Cabernet-Sauvignon, Tempranillo or Chardonnay. Therefore, the reduction of the number of cultivars has caused the forgotten of the old cultivars.

The Balearic Islands, located in the Western part of the Mediterranean basin, are composed by two of the 10 greatest islands in the Mediterranean Sea. Their wines were well known in the world for its high quality since Roman time (Hidalgo 2002), wining several international competitions during nineteen century. The Balearic viticulture has changed over the years; first greenfly named “animaló” appeared (*Haltica ampeloghaga* Guer.; Salvator de Austria 1869), then the powdery mildew (*Erysiphe necator* Schwein) arrived to the islands in 1851 (Salvator de Austria 1869; Ballester 1911), and after that the phylloxera crisis (Hernández Robredo 1903), producing changes in the local cultivars to satisfy the new demand of the consumers.

Due to the water barriers, the vegetal material exchange in the Balearic Islands is only possible by sea, mostly with countries sited around the Mediterranean basin. Therefore, the current gene pool is likely result from plant material exchanges in ports around the Mediterranean basin and from natural crosses occurring on the islands (Prentice et al. 2003). As a consequence, the strategic geographic location of the Balearic Islands and its isolation could be used to disentangle the grapevine movements around occidental Mediterranean basin and the origin of some Balearic cultivars.

The islands isolation has promoted high levels of endemic and genetic divergence (Filippetti et al. 2005; Zerolo and Cabello 2006), which must be clear candidates for conservation (Wilson et al. 2009). Fortunately, new prospecting of grapevine material are rescuing varieties under risk of extinction (Boursiquot et al. 2009; Santana et al. 2010), preserving them in grapevine collections to prevent genetic erosion (This et al. 2006). As a consequence, these old varieties have to be well identified. However, the lack of knowledge of ampelographic descriptions, the grape growing characteristics of antique varieties and grapevine trade as well as the adaptation or change in the name of the grapevines (Cipriani et al. 2010) could have caused the appearance of homonyms and synonyms (Aradhya et al. 2003) implying that sometimes it is not possible to identify the cultivars found in the new prospection (Sladonja et al 2007; Santana et al. 2010). Most of the times, the varieties found in the new prospection are not registered in an official catalogue, thus limiting the opportunity for their conservation, because the growing of these cultivars is not allowed (Salmaso et al. 2008). Nevertheless, some of these varieties show really interesting properties for wine production (Vilanova and Martínez 2007).

The oenological market is a dynamic process that needs to be always adapted to changes and demands on wine market (Bertuccioli 2010), since the wine consumers taste and preferences have changed during the last few years (Lesschaeve 2007). Nowadays, the wine consumers are looking for a new sensorial experience, therefore original wine varieties are starting to be in demand. Therefore, the interest in understanding the origin and genetic diversity of the germplasm rescued in different geographical areas is growing (Cipriani et al. 2010; Schneider et al. 2010). However the potential of most of these wine varieties is unknown. Designations of Origin are required unique and high quality wine varieties which should be linked historically to a geographic area (Meredith et al. 1999; Santiago et al. 2008). At the same time, DOs play an important role in food and wine marketing strategies (Douglas et al. 2001; Skuras and Vakrou 2002), since they are based not only in the geographic area but also in wines quality and originality. Nowadays, Designations of Origin are looking for wine varieties (*Vitis vinifera* L) linked to sites ("authoctonous"), which could provide original and high quality wines, with the aim to increase market possibilities (Bertuccioli 2010). Thus the use of minor varieties could be strong candidates to fill this gap and to satisfy DO requirements, being also one of the last opportunities that minor varieties have to survive in the future. The knowledge of minor varieties possibilities in the changing oenological market is an urgent requirement.

In this study, we analyze 32 accessions of *Vitis vinifera* L. corresponding to 21 different varieties of *Vitis vinifera* L. since some accessions or clones of the same variety were studied (Appendix I). The vegetal material was collected from 1914 to 2000 mainly in the Balearic Islands and nowadays the grapevines are preserved in the *Vitis* Germplasm Bank VGB "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain; Figure 1). However, based on antique bibliography, four accessions of Beba variety were collected from Levante area and Girona, and Pampolat girat variety from Tarragona.

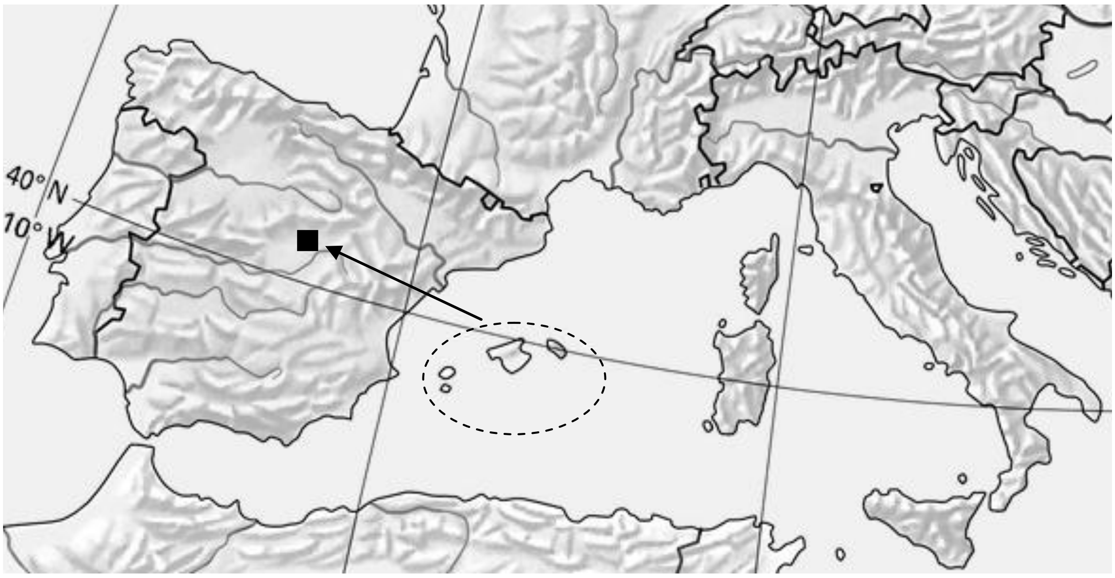

Figure 1. Geographic location on the Western part of the Mediterranean Basin of the Balearic Islands, where the grapevine were mainly collected (circle), and location of the *Vitis* Germplasm Bank VGB "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain) where the grapevines have been preserved (black square)

The main objective of this study was to identify the possibilities of these varieties in the oenological market. Synthesis of historical references, ampelographic descriptions, microsatellite analysis, aromatic grapes characterization and sensory analysis of the made wines have been used for this purpose.

Main objectives

1.- To clearly identify the cultivars found in the antique bibliography providing further information about the grapevines grown and lost in the Balearic Islands from XVII century to nowadays. Chapter 2.

2.- To evaluate the ampelographic descriptions, genetic analysis, agronomic characterization, must variables and phenology of minor grapevine accessions from the Balearic Islands. Chapter 3, Chapter 4 and Appendix II.

3.- To characterize the local cultivars growing in the Balearic Islands using microsatellite analysis, and specifically (i) to clarify cases of synonyms and homonyms of these cultivars with varieties cultivated in other countries; (ii) attempt to know the geographic movement and migration of these varieties, and finally (iii) to establish the genetic relationship between them. Chapter 2, Chapter 3 and Chapter 4.

4.- To analyse the susceptibility of 159 cultivars of *Vitis vinifera* to powdery mildew (*Erysiphe necator* Schwein.) including the cultivars collected in the Balearic Islands. Chapter 5.

5.- To analyse the aromatic potential of 21 grapevines, mainly native from the Balearic Islands. Chapter 6.

6.- To characterize wines made with local cultivars using sensorial description. Chapter 7.

It is important to identify the relationship between cultivar and site to offer to wine consumers minor varieties linked with the “terroir” and with historical background. In this way, antique bibliography is key to understand the evolution of the viticulture in a specific area, relative to the number of cultivars used and growing area. At the same time, this information provides the possibility to correctly identify the varieties found in the new prospection and the grapevine cultivars loss though time.

Other essential point is to provide to vinegrowers and winemakers a well identified material. In grapevine, ampelography is the preliminary method for the clarification of vegetable material (Schneider et al. 2008) and its combination with microsatellite markers allows the correct identification of cultivars (Lopes et al. 1999; Schneider et al. 2010). Microsatellites markers are a powerful tool to distinguish cultivars, clarifying synonyms and homonyms (Lopes et al. 1999; Laucou et al. 2011), and to establish genetic relationships (Boursiquot et al. 2009; Vargas et al. 2009). Chloroplast microsatellite markers are also useful to approach the geographic origin of the grapevine cultivars (Arroyo-García et al. 2006; Imazio et al. 2006) which is usually difficult to establish due to the high material exchange (This et al. 2006).

Finally, agronomic behaviour, the resistance to powder mildew, oenological potential of minor cultivars as well as the acceptance of the wines by experts are required characteristics in order to know the possibilities of minor varieties in the changing oenological market. Typicality of the wines is influenced by a large number of factors, underlining grapevine and vintage (Maitre et al. 2010). Volatile compounds in grapes are influenced by environmental conditions (Ribéreau-Gayon et al. 2006) and they are important as a source of flavour in wines (Franco et al. 2004). In this study all of these factors related to final wine quality are analysed.

It hopes that the information provided in this research would lead to improve the quality of the wines and help to gain more interest of autochthonous varieties, guaranteeing the survival of some of these minor varieties.

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Chapter 2

La mancanza di descrizioni e notizie sopra molti vitigni coltivati anche in regioni viticole importanti, ci costrinse a ricerche lunghe pazienti

(Marzotto N. 1925 Uve da vino: *Descrizione e notizie ampelografiche, viticole ed enologiche dei vitigni più pregiati dell'alta e Media Italia*. Vicenza, Italy: Tip. Commerciale)

Evidence of loss in cultivated grapevines diversity: The example of the Balearic Islands (Spain)

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Abstract

The loss of grapevine varieties over the world has produced an important genetic erosion of the gene pool. This problem is more notable in isolated areas for its particular singularity, characterized by unique specimens, as the case of the Balearic Islands (Spain).

The aim of this study was to quantify the loss of grapevine from XVII century to nowadays; for this purpose it was necessary a thorough investigation to identify the grapevine names found in the antique bibliography. The ampelographic descriptions found in the consulted literature as well as the available information in two grapevine repositories were used to compare the antique descriptions with conserved grapevines nowadays. In this study, possible causes of the change in the Balearic viticulture as several diseases (powdery mildew, flygreen or phylloxera plague), the influence of the Quality Demarcation over evolution of the number of cultivars and vineyard area, the origin of some cultivars as well as the entry of foreign varieties has been discussed.

More than 75% of the cultivars found in the bibliography could be identified. One of the most interesting results was the high grapevine diversity found despite of the small geographic area, being the islands an important stronghold for several cultivars. The greatest change over the Balearic viticulture was dated before the phylloxera plague arrived to the islands contrary to what was thought. The islands were divided in several viticulture areas each one with a reference cultivar. The used methodology in this study was useful to quantify the loss of grapevine variability. Unfortunately, nowadays some of the antique cultivars are lost or under risk of extinction, since an important loss around 50% of grapevine diversity has been quantified through time.

Key words: Conservation genetics, crop evolution, minor varieties, *Vitis vinifera*

Introduction

Based on DNA profiling results, there are around 5,000 grapevine varieties (*Vitis vinifera* L.) around the world (This et al. 2006). This number is decreasing year by year in their natural environmental conditions; evidences of grapevine loosing are obvious in several regions in the world (Ulanovsky et al. 2002; Bessis 2007; Carimi et al. 2010). However some of these grapevine varieties lost in their natural areas are conserved in germplasm repositories. Among the main causes of grapevine variability lost highlights diseases as powdery mildew, phylloxera and the homogenization of international wine market (This et al. 2006; Santiago et al. 2008). A few varieties, such as Chardonnay, Sauvignon blanc or Cabernet-Sauvignon are increasing the vineyard area surface worldwide and replacing the local cultivars (de Mattia et al. 2007; Cipriani et al. 2010), these varieties are also present in most of the European Countries. The introduction of this foreign material induces important changes in the local viticulture, especially in isolated areas which are most sensitive to changes. This is the case of the Balearic Islands where the preservation of local grapevines is critical. As a consequence of water barriers, the vegetal material exchange in the Balearic Islands is only possible by sea, mostly with countries sited around the Mediterranean basin. This isolation has promoted high levels of endemic and genetic divergence (Filippetti et al. 2005; Zerolo and Cabello 2006), producing several unique genotypes (García-Muñoz et al. 2011), in fact these island populations must be clear candidates for conservation (Wilson et al. 2009).

Nowadays, the wine consumers are looking for a new sensorial experience tired from taste of international varieties. Thus original wine varieties are starting to be on demand. At the same time, Quality Demarcations are required original and high quality wine varieties which should be linked historically to a geographic area (Meredith et al. 1999; Santiago et al. 2008). Therefore, the interest in understanding the origin and genetic diversity of rescued germplasm from different geographical areas is growing (Cipriani et al. 2010; Schneider et al. 2010). Unfortunately, the lack of morphological descriptions (ampelography), agronomical characteristics and grapevine trade records of antique varieties implies that sometimes is not possible to identify the cultivars collected in the prospection (Sladonja et al. 2007; Laiadi et al. 2009; Santana et al. 2010).

The first steps toward the identification of varieties linked to an area must be to know the evolution of the viticulture in that area, using as reference the number of cultivar and growing area found in antique bibliography. This information is crucial to identify correctly the varieties found in the new prospection. The principal aim of this work was to identify clearly the cultivars found in the antique bibliography providing further information about the grapevines grown in the Balearic Islands from XVII century to nowadays. Specifically we aimed to (i) to detect the synonyms and homonyms, (ii) to understand the evolution of the number of cultivars and growing area, (iii) to identify the varieties (*Vitis vinifera* L.) connected with the Balearic Islands, and finally (iv) to quantify the varieties loss through time. This information is crucial to identify correctly the varieties found in the new prospection and to analyze grapevine diversity lost. The knowledge of the cultivars observed in the past may provide insights into how to preserve them in the future, giving advices for cultivars conservation.

Material and Methods

Number of cultivars and vineyard area

The material used in this work comprised the information about grapevine found in the antique bibliography; names of cultivars (*Vitis vinifera* L.) and vineyard area from XVII century to nowadays; 438 books dealing with ampelography and viticulture were consulted for this purpose. All the available information as grapevine names, synonyms, homonyms, growing and vineyard surface area as well as ampelographic description found in the literature were collected in a database in base of the cultivation year (if available) or publication year. Each accession name collected in the bibliography was identified when it was possible to assign a “Prime name” (know grapevine name). The “Prime name” was assigned based on the Spanish (BOE 2011), French variety catalogue (IFV 2007) and the *Vitis* International Variety Catalogue (VIVC, www.vivc.batz.de) considering: (1) the ampelographic descriptions, that were used to compare the antique descriptions with the actual grapevines, (2) documents conserved about the studied varieties (i.e. herbarium, drawings, photographs, known homonyms and synonym) in two of the most important grapevine repositories in the world: *Vitis* Germplasm Bank (VGB) “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain) and INRA Grape Germplasm Repository of Domaine de Vassal (Marsellan-plage, France, www1.montpellier.inra.fr/vassal), and (3) published varieties names (i.e. Viticulture books, scientific papers). When a reference was not identified it was not possible to assign a “Prime name", so these cultivars are referenced as “not identified”.

Data analysis

Redundant names per each year were eliminated of the data base to keep only one “Prime name” per variety and year. The relationship between the number of cultivars (identified, not identified and total) and the vineyard area was analyzed using linear regressions with the software XLSTAT, 2009 version.

Results and discussion

Names, synonyms and homonyms

The numbers of grapevine accessions collected in the literature and located in the Balearic Islands were 756. The identified references were 575 (76% of the total) corresponding with 70 different cultivars, since several cases of synonyms were detected (Table 1); in contrast 181 references could not be identified (24%; Table 2). Based on this bibliographical survey, a great grapevine biodiversity was found in the Balearic Islands despite of their small geographic area and isolation. The important number of synonyms found in the bibliography could be due to names adaptations or changes produced trough time (Reisch et al. 1993; Aradhya et al. 2003).

Homonyms were detected for the cultivars refereed in the bibliography as Batista, Vinaté and Giró (Tables 1-2). Batista accessions matched with two different cultivars with hairy leaf (syn. Canari and Luverdon) and with hairless one (not identified variety); Vinaté accessions matched with the red cultivar Bobal and the white one Viñaté; Giró accessions matched with three different cultivars: Mansés de Capdell (syn. Giró and Mancens), Garnacha (syn. Grenache) and Giro sardo (sym. Giró ros). The problem with Giró identification has been pointed out previously (Hidalgo and Candela 1972); however, these homonyms between Giró and Garnacha only exist in bibliography since it is not found in natural conditions or in grapevine repositories nowadays.

Table 1. Prime name, berry colour, and accession names of the Identified varieties in the Balearic Islands. Varieties with * corresponding with the varieties registered in the Spanish or French varieties catalogue (BOE 2011; IFV 2007); † Most antique reference. In bold prime cultivars present in natural conditions in the Balearic Islands nowadays. N: Number of references where the varieties are cited

Prime name	Berry colour	Accession names	† Most antique reference	N
Aleluya blanca	White	Al.leluia blanca, Al.leuier, Aleluya†, Al-leluyas	In 1874, cited in Carretero et al. (1875)	4
Aleluya negra	Black	Al.leluia negra, Al.leluia negra, Aleluies, Aleluya†, Aleluyas	Carretero 1875	9
Alfonso Lavallée / Alphonse Lavallée*	Black	Alfonso lavalle	INDO 1982	1
Aramon*	Black	Planta, Planta rica†	In 1874, cited in Carretero et al. (1875)	4
Argamusa	White	Agamusa, Agremuses†, Argamusa, Argamusas, Argamuses, Argamusses, Argemusa	Salvador de Austria 1869	11
Batista	Black	Batista, Batistes†	Salvador de Austria 1869	20
Beba / Valenci blanco*	White	Calop†, Calop blanco, Calops, Grumer, Jaumes, Mateu, Palop, Pansal, Pansal blanch, Sant Jaume	In 1730, cited in Martí (1978)	33
Beba roja	Rose	Calop rojo, Calops, Grumè, Polop áspero†	Varcárcel 1765	6
Bobal*	Black	Boal, Bobal†, Vinate	Carretero 1875	9
Cabernet-Sauvignon*	Black	Cabernet sauvignon	BOE 1995	1
Callet*	Black	Callet	Satorras 1892	18
Cañorroyo	White	Cañarroyo	INDO 1982	1
Carcajolo / Monvedro*	Black	Beni Salem, Beni-salem, Beni-Salem, Beni-salem de Majorque†, Binissalem	Odart 1845	7
Cardinal*	Red	Cardinal	INDO 1982	2
Cayetana Blanca / Pardina*	White	Jaen	Estelrich 1903	1
Chardonnay*	White	Chardonnay	BOE 1998	1
Chasselas cioutat*	White	Peu de rata	García-Muñoz et al. 2011	1
Cinsaut*	Black	Cinsault, Cinsaut†, Sinsó	Satorras 1892	6
Corazón de cabrito / Cornichon blanc*	White	Maimons blancs, Memeles de vaca†, Teta de Vaca blanca	Salvador de Austria 1869	1
Eperó de Gall	Black	Eperó de gall, Esperons de gall†	Salvador de Austria 1869	3
Excursach / Murescu	Black	Cursac, Escumacs, Escursac, Escursach†, Excursach,	In 1842, cited in Ferrer (1999)	13
Fernandella	Black	Fernandella†, Ferrandella, Ferrandilla	Aragó 1871	8
Fogoneu*	Black	Fogonen, Fogonén, Fogoneu, Fogonéu, Fogoneu francés, Fogoneus†	Salvador de Austria 1869	30
Gafarró	Black	Gafarró	Anonymous 1978	1
Garnacha Tinta / Grenache*	Black	Garnacha†, Garnacha negra, Garnatxe, Giró, Girons, Granacha, Granatxa	Vargas Ponce 1787	23
Garnacha Tintorera / Alicante Henri Bouschet*	Black	Alicante, Híbridos Bouchet, Tintorer†	In 1874, cited in Carretero et al. (1875)	8
Giró / Mancens	Black	Chances de Capdell, Giró, Mancés†, Manset, Mansés de Capdell	In 1874, cited in Carretero et al. (1875)	11
Giró Ros / Giro sardo*	Rose	Giró, Girons†, Giró ros	Salvador de Austria 1869	4
Gorgollassa*	Black	Gargallosa, Gargollasa, Gargollassa, Gargollosa, Gorgollasa, Gorgollassa, Gorgollasses†, Gorgollosa, Gorgolloso	In 1856, cited in Anonymous (1878a)	30
Hebén / Gibi	White	Panses	García de los Salmones 1914	1

Prime name	Berry colour	Accession names	† Most antique reference	N
Macabeo / Macabeu*	White	Macabeo	Forteza 1944	2
Malvasia aromatica / Malvasia de Sardegna*	White	Malvasia, Malvasía†, Malvasía de Banyalbufar	In 1617, cited in Agustí (1722)	24
Mandón / Garró*	Black	Galmeta, Gamete, Garró, Mandó	García de los Salmones 1893	5
Mansés de Tibbus	Black	Mancés d´en Tibus, Mancés d´en tibús, Mances den tibús†, Mansés de Tibbus, Mansesos de en Tibbus	Carretero 1875	6
Manto Negro*	Black	Cabelis, Manto negro†	Satorras 1892	12
Mazuela / Carignan*	Black	Carignan†, Cariñena	Anonymous 1889	6
Merlot*	Black	Merlot	BOE 2001	1
Merseguera*	White	Cañavella	García de los Salmones 1914	1
Mollar Cano / Negra mol*	Black	Mollar	Estelrich 1903	1
Monastrell / Mourvèdre*	Black	Mandó, Monastrell†, Monestrell, Morrastrell	Salvador de Austria 1869	9
Moscatel de Alejandría / Muscat d´Alexandrie*	White	Momona de Pollenza, Moscatel†, Moscatel de Málaga, Moscatel de Mallorca, Moscatel romano, Moscateles, Moscatell, Moscatell romá	In 1617, cited in Agustí (1722)	27
Moscatel de Grano Gordo Rosado / Muscat à petit grains roses*	Rose	Moscatel†, Moscatel rosado, Moscatel vermeill	Salvador de Austria 1869	6
Moscatel de Grano Menudo / Muscat à petit grains blancs*	White	Moscatel menudo, Moscatel menudo blanco, Moscatel menut†	Carretero 1875	6
Ohanes*	White	Ohanes	Estelrich 1903	1
Palomino Fino / Listán Blanco*	White	Jerez, Listan, Ojo de liebre, Ull de llebre†, Ulls de llebra	Salvador de Austria 1869	7
Pampolat girat	Black	Botget de parmpoml lluent, Cruxent, Cruixen, Pampal gira†, Pampol girat, Pampolat	Odart 1845	10
Pardillo*	White	Pardillo	INDO 1982	1
Parellada*	White	Montana, Montaña, Montenach, Montona†, Montonas, Montonec, Montonés, Multonac, Multonachs, Muntona, Muntone, Parellada	In 1816, cited in Albertí and Rosselló (2007)	24
Pedro Ximénez*	White	Pedro Giménez, Pedro Jiménes, Pedro Jiménez†, Pedro Ximénez, Pedro-Ximinez	Satorras 1878	8
Pensal Blanca / Moll*	White	Moll, Pansal blanco, Pensal blanc, Pensal blanco†, Penzal blanco, Premsal blanc	Estelrich 1903	11
Pepita de oro	White	Pepita de oro	INDO 1982	2
Picapoll Blanco / Piquepoul blanc*	White	Picapoll blanco, Picapolla albilla†	Despuig 1784	2
Pinot noir*	Black	Pinor noir	BOIB 2005	1
Planta Fina de Pedralba / Farana*	White	Alicante blanco, Farana, Farrana, Ferrana, Ferrana blanca, Majorcain†, Majorquen, Majorquen blanc, Majorquin, Mallorqui, Mallorquí, Mallorquin, Mayorcain, Mayorcain blanc, Mayorquen, Mayorquen blanc, Mayorquin, Planta, Plantes	Odart 1845	23
Quigat	White	Cagat, Cagats†, Masacamps, Quigat, Quijat	Salvador de Austria 1869	15
Riesling*	White	Riesling	BOIB 2005	1
Roseti / Dattier de Beyrouth*	White	Roseti	INDO 1982	1
Sabaté	Black	Sabaté, Sabater, Sabaters†	Salvador de Austria 1869	20
Santa Magdalena	White	Juanillo†, Magdalena, Santa Magdalena, Temprana	Carretero 1875	9
Sumoll Tinto*	Black	Sumoll	García de los Salmones 1914	5

Prime name	Berry colour	Accession names	† Most antique reference	N
Syrah*	Black	Syrah	BOE 2003	1
Taferielt / Farana noir	Black	Ferrana negra	García de los Salmones 1914	1
Tempranillo*	Black	Tempranillo, Tinto ribera, Ull de llebre†	García de los Salmones 1914	3
Teta de Vaca Tinta / Ahmeur Bou Ahmeur	Red	Maimó, Maimons negres†	Salvador de Austria 1869	2
Trepat*	Black	Parrel	Anonymous 1907	1
Valenci Tinto / Valensi noir*	Black	Balancí negre, Beba negra, Calop negro, Calop tinto, Grumés rojos	Salvador de Austria 1869	4
Valent Blanc	White	Valent blanch, Valent blanco, Valent-blanc, Valents blancs†	Salvador de Austria 1869	15
Valent Negre	Black	Valent negre, Valent negro, Valent-negre, Valents negres†	Salvador de Austria 1869	11
Viñaté	White	Panse valenciano, Vinaté, Vinater, Vinaters†, Vinatés, Viñater	Salvador de Austria 1869	15
Xarello*	White	Cartuxa†, Pansa valenciana†, Xarel.lo, Xarel-lo, Xarelló†, Xarello blanco, Xarelo	García de los Salmones 1914	7

Some of not identified varieties were referenced to a color as Blanquet, Bermell and Negrillo (which means white, rose and black respectively), or to a generic names as Inglés (from England), López (a very common Spanish surname) or Pansal (which means “to do raisin”; Table 2). In some cases not identified varieties showed different grape colour which did not match with nowadays conserved grapevines (e.g. Argamusa, Esperó de gall, Moll, Quigat; Tables 1-2). The presence of not identified cultivars could be caused by identity lost or new crosses produced over the years. Our previous results validate this second explanation since some unknown varieties found in the last prospection did not match with other varieties around the world, therefore these varieties could be considered unique genotypes (García-Muñoz et al. 2011). In any case, the information lost about not identified references makes impossible their identification until now.

Table 2. Prime name, berry colour, and accession names of the not identified varieties in the Balearic Islands

Accession names	Berry colour	Reference
Aigomel	White	Satorras 1878
Alaró	Not specified	Anonymous 1886
Alicantí	Not specified	Forteza 1944
Argamusa	Black	Hidalgo 1991
Asturell	Black	García de los Salmones 1914
Asturell	White	García de los Salmones 1914
Babarrés	Not specified	Anonymous 1889
Barbaresus	White	García de los Salmones 1914
Barroves	White	Carretero 1875
Batista	Black	Anonymous 1889
Batzoles	Black	Salvador de Austria 1869
Beberrés	White	García de los Salmones 1914
Bermell	Not specified	Rodríguez Navas 1904
Bermell	Not specified	Sánchez 1915
Blanquet	White	García de los Salmones 1914

Accession names	Berry colour	Reference
Bragat	Black	Carretero 1875
Bregats	Not specified	Carretero et al. 1875
Calop moscatell	Not specified	Griera 1935
Calop-Giró	Not specified	Sánchez 1915
Calop-Giró	Not specified	Anonymous 1978
Calop-Pauzal	Not specified	Sánchez 1915
Cariavella	White	Carretero 1875
Castellanas	Not specified	In 1617, cited in Agustí (1722)
Champany	Not specified	Forteza 1944
Ciusant	Not specified	Ballester 1910
Ciusant	Not specified	Ballester 1911
Cruixó	Not specified	Griera 1935
Duricie	White	Carretero 1875
Esperó de gall	White	Carretero 1875
Estorell	Black	García de los Salmones 1914
Foguan	Not specified	Anonymous 1978
Font negro	Black	García de los Salmones 1914
Formigons	Black	Salvador de Austria 1869
Furmigó	Not specified	Carretero et al. 1875
Furmigó	Black	Carretero 1875
Giró	Rose	In 1616, cited in Alcover and Moll (2001)
Giro	Not specified	Despuig 1784
Giró	Not specified	In 1816, cited in Albertí and Roselló (2007)
Giró	Not specified	Weyler y Laviña 1854
Giró	Not specified	Anonymous 1861
Giró	Not specified	Anonymous 1878b
Giró	Not specified	Salvador de Austria 1869
Giró	Not specified	In 1889, cited in Albertí and Roselló (2007)
Giró	Not specified	Ballester 1911
Giró	Not specified	García de los Salmones 1915
Giró	Not specified	Forteza 1944
Giró	Not specified	Marcilla 1949
Giró	Black or rose	Griera 1965
Giro pansal	Not specified	Estelrich 1877
Gorch	White	García de los Salmones 1914
Gorgs	Black	Salvador de Austria 1869
Gorru	Not specified	Griera 1935
Gots	Black	García de los Salmones 1914
Graperas	Not specified	Forteza 1944
Grapesa	Black	García de los Salmones 1914
Grech	Black	García de los Salmones 1914
Grech	White	García de los Salmones 1914
Imperial	White	García de los Salmones 1914
Inglés	Not specified	Satorras 1892
Jaumillos	Not specified	Forteza 1944

Accession names	Berry colour	Reference
Juanillo	White	García de los Salmones 1914
Juanillo	Not specified	Marcilla 1949
Juanillo	Not specified	Anonymous 1950
Juanillo	Not specified	Hidalgo and Candela 1972
Juanillos	White	Salvador de Austria 1869
Leuries	Not specified	Salvador de Austria 1869
Llora	Not specified	Anonymous 1889
Lloras	Black	García de los Salmones 1914
Lloreta	Black	García de los Salmones 1914
Llorete	Not specified	Forteza 1944
Llurguei	White	Carretero 1875
Lopez	Black	Carretero 1875
Lopez	Rose	Satorras 1878
Lopez	Black	Anonymous 1889
Lopez	Rose	Satorras 1892
López	Black	Ballester 1910
López	Not specified	Ballester 1911
López	Black	García de los Salmones 1914
Loretes	Black	Salvador de Austria 1869
Maiblanch	Black	Carretero 1875
Mandó	Not specified	Forteza 1944
Mandó	Not specified	Anonymous 1950
Mandó	Not specified	Hidalgo and Candela 1972
Manzanilla	Black	García de los Salmones 1914
Mapisco	Black	Carretero 1875
Marol	Black	Carretero 1875
Mateu	Black	García de los Salmones 1914
Moll	Black	García de los Salmones 1914
Mollá	White	Carretero 1875
Mollá	Not specified	In 1885, cited in Pastor Sureda (1980)
Mollá	Not specified	Satorras 1892
Mollar	Blanco	Satorras 1878
Mollar	Not specified	Despuig 1784
Mollar	Not specified	Anonymous 1861
Mollar	Not specified	Salvador de Austria 1869
Mollar	Not specified	Anonymous 1878b
Mollar	Not specified	Anonymous 1889
Mollar vert	Not specified	Salvador de Austria 1889
Molls	Black	Salvador de Austria 1869
Moltona	Black	García de los Salmones 1914
Montona	Not specified	Weyler y Laviña 1854
Montona	Black	Salvador de Austria 1869
Montona	White	Satorras 1878
Montona	White	Ballester 1911
Mora	Black	García de los Salmones 1914

Accession names	Berry colour	Reference
Morastel	Black	García de los Salmones 1914
Morete	Not specified	In 1842, cited in Ferrer (1999)
Moscatell	Not specified	In 1382, cited in Canals i Frontera (1989)
Muntona	Not specified	Despuig 1784
Mustrell	Black	García de los Salmones 1914
Negrelles	Black	Salvador de Austria 1869
Negrillo	Not specified	Satorras 1892
Ojo de liebre	Not specified	Ballester 1911
Padre	White	Carretero 1875
Padré	White	García de los Salmones 1914
Pampal	Not specified	Viala and Vermorel 1903
Pampals	Not specified	Viala and Vermorel 1903
Pampals	Not specified	Viala and Vermorel 1909
Pampégat	Not specified	Viala and Vermorel 1909
Pampol roda	Not specified	Salvador de Austria 1869
Pampol rodal	Not specified	Casas de Mendoza 1857
Pampol rodal	Rose	Rodríguez Navas 1904
Pampol rodat	Not specified	Vargas Ponce 1787
Pampol rodat	Not specified	Varcárcel 1791
Pampol rodat	Not specified	Weyler y Laviña 1854
Pampol rodat	Not specified	Anonymous 1878a
Pampol rodat	Not specified	Abela y Sáinz de Andino 1885
Pampol rodat	White	Anonymous 1889
Pampol rodat	White	Satorras 1892
Pampol rodat	White	Ballester 1910
Pámpol rodat	White	Matons 1928
Pàmpol rodat	White	Salvador de Austria 1869
Pampol rosat	Not specified	Despuig 1784
Pampol rosat	Not specified	In 1816, cited in Albertí and Roselló (2007)
Pampol rosat	Not specified	Anonymous 1861
Pampol rosat	Not specified	Salvador de Austria 1869
Pampol rosat	White	Carretero 1875
Pampol rosat	Not specified	Estelrich 1877
Pampol rosat	Not specified	Salvador de Austria 1889
Pampolrodat	White	Satorras 1878
Pampol-rosat	Not specified	In 1885, cited in Pastor Sureda (1980)
Pansa	White	Griera 1965
Pansal	Black	García de los Salmones 1914
Pansal	Not specified	García de los Salmones 1915
Pansal blanch	White	Satorras 1878
Pansal blanch	White	Anonymous 1889
Pansal blanch	White	Satorras 1892
Pansal blanch	White	Ballester 1910
Pansal blanch	White	Carretero et al. 1875
Pansal negre	Black	Carretero et al. 1874

Accession names	Berry colour	Reference
Pansal negre	Black	Satorras 1878
Pansal negre	Black	Anonymous 1889
Pansal negre	Black	Satorras 1892
Pansal negre	Black	Hernández Robredo 1903
Pansal negre	Black	Ballester 1910
Pansal negre	Black	Ballester 1911
Pansal negro	Black	Anonymous 1878b
Pansals	Black	Salvador de Austria 1869
Pansé	Not specified	Forteza, 1944
Panses	Black	Salvador de Austria, 1869
Panzal	White	Pacottet 1928
Parra borda	Not specified	Barceló y Combis 1879
Pauzal	Not specified	Sánchez 1915
Pensal blanch	White	Carretero 1875
Pensal negre	Black	Carretero 1875
Picapol	Not specified	Satorras 1892
Planta	Not specified	Estelrich 1877
Ponzal	Not specified	Anonymous 1978
Quigat	Black	Ballester 1910
Quigat	Black	Ballester 1911
Rosada	Black	García de los Salmones 1914
Sabaté	White	Homar Solivellas 1978
Tarrés	Black	Salvador de Austria 1869
Ullada	Not specified	Satorras 1892
Ullades	Black	Salvador de Austria 1869
Ulls de Llebra	Not specified	Carretero et al. 1875
Vennaçza	Black	Carretero 1875
Vernatxa	Black	García de los Salmones 1914
Vinaté	Not specified	Forteza 1944
Vinater	Not specified	Ballester 1911
Vinatés	Not specified	García de los Salmones 1915

Evolution and relationship between the number of cultivars and growing area surfaces

The number of cultivars identified was always greater than the number of varieties not identified in all the studied years (Figure 1). Nowadays, there are 36 identified cultivars in the Balearic Islands (Figure 1) which matched mainly with the varieties allowed in several Demarcations of Quality. The number of identified cultivars was not related with the vineyard area (r^2=0.09; p=0.094), however the not identified accessions as well as the total number of cultivars were significant related with the vineyard area (r^2=0.30; p=0.001; r^2=0.18; p=0.013; respectively). The vineyard area has decreased significantly through years (r^2=0.63; p<0.001).

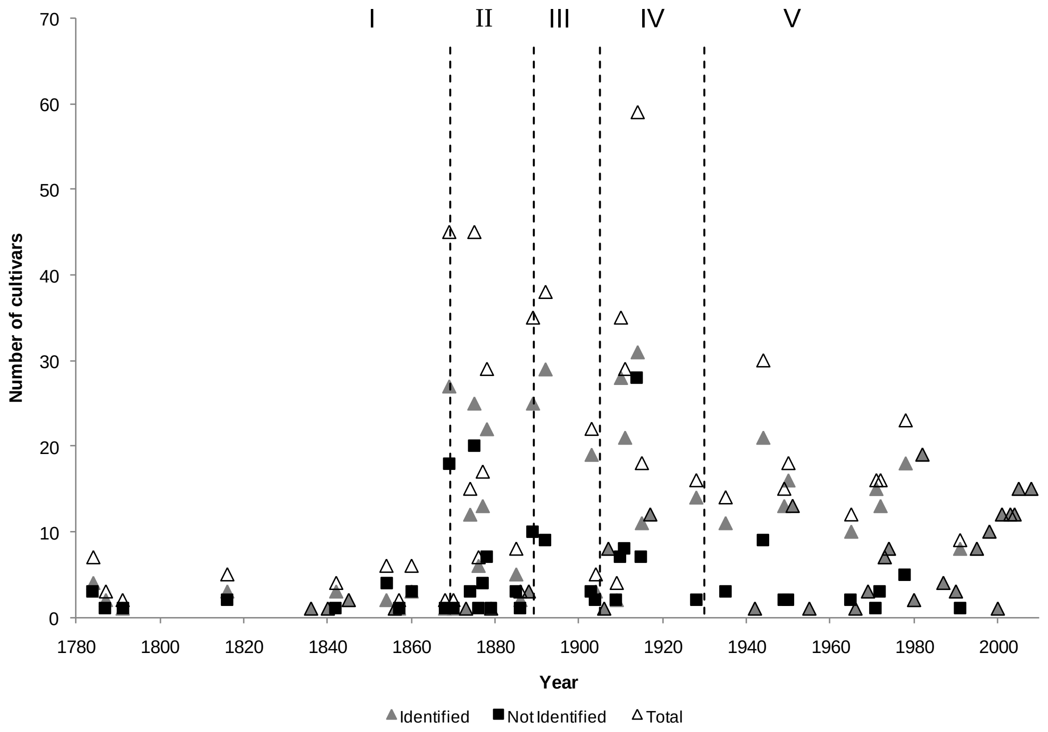

Figure 1. Number of cultivars Indentified, Not Identified and Total per year found in the bibliography. Each of the five periods is noted by roman numbers

Taking in consideration the tendency of the curve and the historical facts, the vineyard area evolution could be grouped in 5 periods (Figure 2).

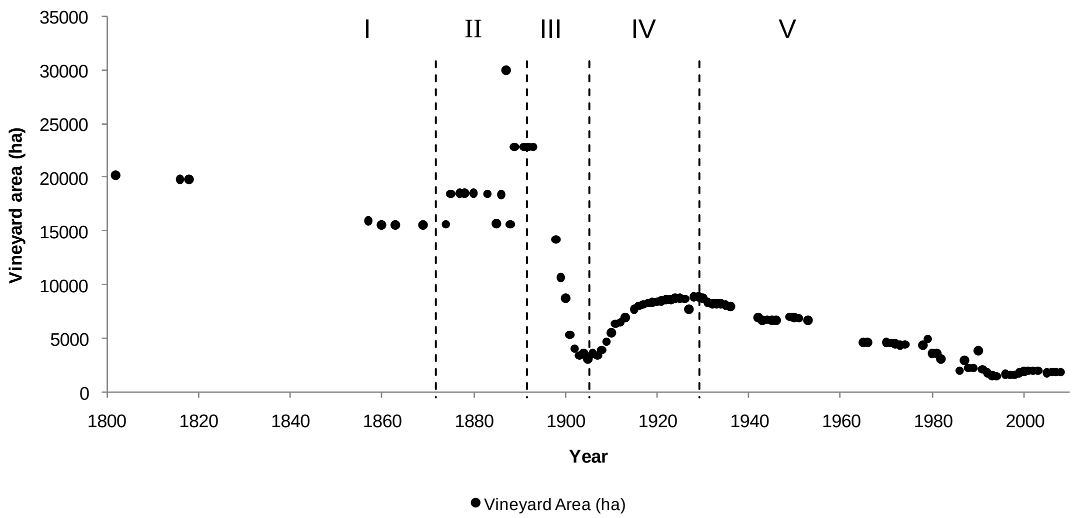

Figure 2. Vineyard area (in hectare) per year found in the Bibliography. Each of the five periods is noted by roman numbers

In the first period, from the beginning of the study until 1870, the number of cultivars identified, not identified and total was greater in 1869 (27, 18, 45 respectively; Figure 1). The vineyard area surface decreased from 20,192 ha to 15,543 ha, related with the greenfly named “animaló” (*Haltica ampeloghaga* Guer.; Salvator de Austria 1869) and the introduction in 1851 of powdery mildew (*Erysiphe necator* Schwein.; Salvator de Austria 1869; Ballester 1911). These diseases caused a drastic surface area reduction of several cultivars, especially in the case of most appreciated as Escumacs (syn Excrusach), Garnacha (syn. Grenache), Giró, Malvasía, Mollar (Salvator de Austria 1889) and Montona (Satorras 1892; Ballester 1911). As a result, during this period there were attempts to recover the previous grapevine areas of these varieties, in example Antonio de Cotoner tried to recover the vineyard area of Malvasia importing material from Malta (Salvador de Austria 1869).

Among the second period (1871-1891), the vineyard area surface increased up to 22,833 ha mainly caused by the appearance in 1863 of the phylloxera plague in France. Then Spain and specifically the Balearic Islands improved the wine commercial relationship with France to export their products (López y Camuñas 1878), that was promoted by a trade agreement between France and the Balearic Islands in 1882 (Ripoll 1974). As a consequence, the local cultivars were changed to satisfy the French demand (Ballester 1911), choosing cultivars more productive as Callet and Manto Negro instead of less productive but more quality local cultivars as Gorgollasa, which almost disappeared. In this second period the number of cultivars identified, not identified and total was greater in 1875 (25, 20, 45 respectively; Figure 1). In this year, not identified varieties achieved one maximum (20 cultivars), this might be caused by the high number of imported cultivars. In fact, Antonio Cotoner and other vine growers had imported vegetal material from France, Malta and South of Spain (Salvator de Austria 1869). As a consequence, the first references to French varieties appeared, as Cinsaut (Satorras 1892) or Aramon which was imported under the Spanish name of Planta Rica (meaning delicious plant) by Paulino Verniere (Carretero et al. 1875; Estelrich 1877). Garnacha (syn. Grenache) was imported again from Catalonia (Spain; Carretero et al. 1875), since the powdery mildew had caused a considerably decrease over its growing area in the islands. During this second period some local cultivars as Callet and Manto Negro were chosen for its high production of grapes and have been conserved to nowadays. Based on these results, the most important events that changed the viticulture of the Balearic Islands were dated in this period, before the phylloxera plague arrived to the islands.

Despite of the measures taken, the phylloxera plague was officially declared the June 4th of 1891 in the Balearic Islands (Pou y Bonet 1880). Consequently, in the third period (1892-1905) the vineyard area surface decreased drastically to 3,075 ha until 1905. From 1906 to 1929 (fourth period) the vineyard area increased to 8,826 ha, but never reached the previous cultivated area before the phylloxera plague. The completely re-plantation of French vineyard (García de los Salmones 1915) and the changes in land use, many growers decided to plant tomatoes (Albertí and Rosselló 2007) and almonds instead of vineyard (Junta Consultiva Agronómica 1915), were the causes of vineyard area contention. The long recovery period for vineyard could be due to the bad results in the grafting (Martínez Rosich 1900) and the long time taken to find compatible rootstocks with the calcareous soils (Pacottet 1928; Forteza 1944). In this period, in 1914 the total number of varieties increased up to 59, being the maximum of the identified, not identified and total varieties (31, 28 and 59 respectively). The main cause of the maxima could be the research of new material for the vineyard recovery, used to test the compatibility between several varieties and rootstocks imported from the Iberian Peninsula. Also among this period, a rigorous studied was done simultaneously in all the Spanish regions with a scientific objective to prevent the grapevine diversity loss caused by the phylloxera plague, so numerous varieties were identified and collected in natural conditions in Spain. These varieties were conserved in grapevine repositories, therefore they have survived until nowadays.

During the fifth period, from 1930 to nowadays, the vineyard area has decreased to 1,795 ha. The decreases of vineyard area from 1931 could be caused by three factors: first the Spanish Civil War (1936-1939), second the touristic boom around 1960 and third the wine market expansion. In contrast, there were slight increases of vineyard area from 1987 to 1990 and in 1997, around the years when the two Denominations of Origin were created (Binissalem in 1991 and Pla i Llevant in 1999; Figure 1). The creation of the Denominations of Origins seems to influence positively on the vineyard area. However, the appearance of Denominations of Origin reduced the number of local cultivars planted. This is clearly shown in the lack of international varieties found in the bibliography in 1982, whereas 25 years later, in 2008, six cultivars out of the 15 cultivars allowed in the Balearic Islands Quality Demarcations are international varieties (Cabernet-Sauvignon, Chardonnay, Merlot, Pinor noir, Riesling and Syrah). Therefore, the homogenization of the wine marker is decreasing the local cultivars over the years, as it is reported in other viticulture areas around the world (This et al. 2006; de Mattia et al. 2007; Carimi et al. 2010).

Cultivars connected with the Balearic Islands

The most antique grapevine references were the varieties “Moscatell” in 1382 (Canals i Frontera 1989), “Giró” in 1616 (Alcover and Moll 2001); “Castellanas” and Malvasía (prime name Malvasía aromatic, synonym Malvasía de Sitges) in 1617 (Agustí 1722); Calop (prime name Beba, Valenci Blanco) in 1730 (Martí 1978); “Mollar”, “Muntona”, “Pampol rosat” and Picapolla albilla (prime name Picapoll Blanco) in 1784 (Despuig 1784); Garnacha (syn. Grenache) and “Pampol rodat” in 1787 (Vargas Ponce 1787; Table 1).

Among the identified cultivars, the most referenced cultivars were Beba (prime name Valenci Blanco) with 33 references, followed with 30 references appeared the cultivars Fogoneu placed in Porreras, Felanitx and Manacor (Estelrich 1877), and Gorgollasa located in the central part of the Majorca Island (Estelrich 1877) and Inca (Anonymous 1878a). Moscatel (prime name Moscatel de Alejandría) appeared in 27 references. Montona variety (prime name Parellada) was referenced 24 times and was located in Pollensa (Despuig 1784; Anonymous 1878a), and finally, Malvasía variety (prime name Malvasía aromatic, synonym Malvasía de Sitges) shared the same number of references which was placed in Esporles (Despuig 1784) and Banyalbufar (North of Majorica

Island; Anonymous 1878a; Ripoll 1974; Table 1). In base of these results, we hypothesize that the Balearic Islands were divided in several viticulture regions, each one with a principal cultivar such as Malvasía in Banyalbufar (Noth of the Majorca Island), Montona in Pollensa (Northwest of Majorca Island), Gorgollasa in central of Majorca Island and Fogoneu in South-west of the Majorca Island. Among the not identified varieties, Giró, Pampol rodat and Pampol rosat were the most referenced cultivar names (Table 2).

Although the origin of Montona variety (prime name Parellada) is unknown, the Croatian (Montona, Istria actual Croatia) or Italian origin has been suggested (Oliver Moragues 2000), however other theory relates its name with the adaptation to the foot of the mountains environmental conditions. The origin of Beba (prime name Valenci Blanco) and Excursach (synonymy Murescu) seems to be related with the Moors during the Islam expansion (García-Muñoz et al. 2011).

There are two cultivar names that seems to be specifically related with the Balearic Islands; the black cultivar Binissalem (name of a village sited in the central of the Majorca Island) matched with the variety Carcajolo (syn. Monvedro) and the white one Mayorquin (from the Majorica Island) matched with Planta Fina de Pedralba (syn. Farana). These results were corroborated in VGB “Finca El Encín” and INRA Domaine de Vassal repositories, based on the bibliographical references, ampelographic descriptions and microsatellite data.

Binissalem (principal names Carcajolo, Monvedro) was located in Spain (the Balearic Islands, Extremadura and Catalonia), Algeria, Corsica, Sardinia, Portugal (around Lisbon area, Algarve and Alentejo) and Australia. Although its origin is unknown the North-African origin has been suggested by Pulliat (1898). Our results suggest that this cultivar could come to Majorca Island in VIII century when Majorca was brought by the Arab, and then kept to Portugal by the sea routes during the XIV century. In fact, a sea route existed in 1301 which connected Majorca Island with the Western Mediterranean and the Atlantic sea; there are references indicating that Majorcan people trip to Lisbon (Portugal) in the year 1340 (Fulgosio 1870; Abulafia 1996).

The last cultivar that could be connected with the Balearic Islands is the white variety Mayorquin (principal names Planta Fina de Pedralba and Farana), which was located in France (Provence), Spain (León, the Balearic Islands and Cataluña) and Algeria. The origin of this cultivar is unknown but it seems to have an Oriental origin since the Balearic Islands were a strategic point in several sea routes from the East of the Mediterranean basin to the North of Africa (Abulafia 1996).

Varieties loss through time

The grapevine diversity has been drastically reduced in the Balearic Islands. Nowadays, only 36 varieties out of 70 identified varieties through time are found in natural conditions (51.4%), six of them (16.7%) are international varieties allowed in the Demarcations of Quality, whereas nine are table varieties (25.0%). These results imply that we have almost lost a half of the identified varieties in natural conditions. In addition, none of the not identified cultivars has been found in natural conditions or grapevine repositories, perhaps we have lost all of them, including the cultivar which showed a grape color variation with respect to the conserved cultivars. The used methodology in this study was useful to quantify the loss of grapevine variability.

An interesting result was the case of the not identified cultivar related to “Pampolat” designations which are not found in natural conditions. In VGB “Finca El Encín” an accession with Pampolat girat name exists. This is the only cultivar that could be related with these references, however Pampolat girat is a black variety and the references to the “Pampolat” designations are white or rose.

The varieties connected with the Balearic Islands, Mayorquin (prime name Planta Fina de Pedralba, Farana) and Benissalem (prime name Carcajolo, Monvedro), are not present in the Balearic Islands however are found in natural condition in other countries (i.e. France, Algeria), so their preservation seems to be guaranteed at the moment. However, Argamusa, Quigat and Sabaté, which are unique genotypes not found out of the Balearic Islands (Spain), are reducing their growing area year by year. Argamusa and Sabaté were considered with lower quality for wines during XIX century, recommending their no cultivation (Carretero et al. 1875; Anonymous 1889), in fact this could be the reason because these cultivars are minor varieties nowadays. Fortunately, the preservation of these minor varieties in grapevine repositories is saving the dramatic loss of genetic variability.

Particularly interesting is the case of Gorgollasa variety which was an important cultivar cited in central of the Majorca Island, although nowadays it is in risk of extinction. It was no longer grown from the French phylloxera plague because of its low production, despite their wines obtained several international prizes towards the end of the XIX century (Anonymous 1878b). Actually the Quality Demarcation Binissalem (Balearic Islands, Spain) is trying to recover this antique cultivar.

Fortunately, several recent works (Albertí and Rosselló 2007) and new prospection are rescuing some unique cultivars as Valent negre and Valent blanc (García-Muñoz et al. 2011), which are preserved in several grapevine repositories for their preservation.

Conclusions

This is the first time that using all the information available we could be able to approach accurately the number of cultivars lost and their evolution in relation with the changes over the viticulture of a specific area. The most important events that changed the viticulture of the Balearic Islands were dated before the phylloxera plague arrived to the islands. The creation of the Demarcations of Quality has stabilized the number of cultivars, whereas the new interest on antique cultivars to satisfy the new wine consumers demand has a positive effect over the antique grapevines conservation.

The grapevine diversity found in the Balearic Islands was very high despite of their small geographic area with 70 varieties identified. Most of the grapevine names found in the antique bibliography have been identified and fortunately some of them have been conserved until nowadays as Callet, Manto Negro or Gorgollassa. However, several grapevine names found in the bibliography did not match with varieties cultivated nowadays and remains unknown.

The methodology used in this study might be of great interest to quantify the loss of grapevine variability in other viticulture areas. The loss of grapevine diversity is evident; we have lost the half of the identified cultivars growing in natural conditions in approximately 300 years. The high rate of grapevine loss needs to provide roles of conservations. Special attention is recommended for the unique genotypes not found around the world out of the Balearic Islands as Argamusa, Sabaté or Quigat varieties which are decreasing their growing area. The rescue of the cultivars found in the new prospection contributes to the conservation of the grapevine genetic variability. They must to be preserved in grapevine resources before the loss of these cultivars is irreversible.

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Chapter 3

Hopeful

Ampelography: an old technique with future uses, the case of minor varieties of *Vitis vinifera* L. from the Balearic Islands

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Abstract

The aim of the present work was to evaluate the ampelographic descriptions, genetical analysis, agronomic characterization, must variables and phenology of 27 minor grapevine accessions from the Balearic Islands (Spain). The influence of different agronomic variables, occasional climatic phenomena (hailstorm) and the ampelographer's experience were studied.

Grapevine accessions were analysed using 58 OIV qualitative and quantitative descriptors and 6 SSR. Ampelography is a good preliminary technique for the clarification of vegetable material, 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 the flesh (OIV-235) were the most difficult characters to distinguish by ampelographers. In spite of the greater similarity found among varieties studied, there are certain strong key characters for identification of these varieties (OIV-225, OIV-084, OIV-053, OIV-004). In addition, the ampelographic descriptions, agronomic parameters and phenology were influenced by occasional climatic phenomena (hailstorm).

The morphological and molecular characterizations of 27 accessions recollected in Balearic Islands (Spain) were allowed to validate the ampelographic description method and grouped them in 17 vine varieties. The genetic analysis showed that Beba blanca as a possible somatic mutant from Beba roja. The hailstorm increased the vegetative period, specially affected at mature leaf, bunch, agronomic characteristics and the composition of the must.

The present work characterize, for the first time, the ampelographic and molecular profiles of these minor varieties. The study showed potential and interest of the cultivars suggesting that their utilization may be important for the farmers.

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

Introduction

Until a few years ago, ampelography has been the main method used for describing and then identifying vine varieties (Schneider et al. 2008). Studies of ampelography using well defined OIV, UPOV and UPGRI official descriptors supplied insights into clarification of vegetable material (Pavek et al. 2003). The notations on the phenotypic characteristics of different cultivars taken by important ampelographers over one hundred years ago (Odart 1874) have been confirmed years afterwards by genetics (Schneider et al. 2008), validating ampelography as a preliminary method for the clarification of vegetable material. However, in the last few years, confusing vegetable material due to a lack of ampelographic knowledge has led to legal and commercial controversies.

In the last years molecular markers, and specially microsatellites, has became an essential tool for the identification of grape varieties (This et al. 2004). They do not show the problem of interaction with the environment or subjective interpretations, often attributed to some ampelographic descriptors. And they make easier the exchange of results between laboratories by comparison of genetic profiles. However differences based on somatic mutations such as the colour of the berry, only can be certified if the material is evaluated in the field (Fatahi et al. 2003). Then, for clonal verifications the microsatellite technique is not valid (Cabezas et al. 2003). Therefore, it is also 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, since 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 extincted (de Mattia et al. 2007). However, nowadays wine consumers are demanding new products which increased the interest of winemakers and researchers on 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 must been studied as a pre-requisite in order to include them in a germplasm collection (Alleweldt and Dettweiler 1989). Simultaneously, ampelographic description is compulsory 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 used them. At the same time, the ampelographic technique is cheaper and more accessible than the microsatellite where a specialised laboratory is required. So, the morphological description of the vine varieties could be used to prevent possible mistakes in the plantations, nurseries or vine growers, and can be later verified by the microsatellite techique. The combination of molecular markers and ampelographic descriptions irrefutably certifies the varietal identification in a more thorough way and leads to more reliable and objective results (Hinrichsen et al. 2001; Santiago et al. 2005).

Some authors suggest that atmospheric changes have an influence on morphological characteristics (Alleweldt and Dettweiler 1989; Cunha et al. 2009) and vineyard management creates changes in ampelographic descriptions (Bowers et al. 1993). Because of that, morphological descriptions have been discredited and considered less reliable because it can be confused with 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 ampelographyc characterization. In this work we have analyzed 27 accessions from Balearic Islands (Spain) vine varieties (*Vitis vinifera* L.), where previous morphological studies have not been performed. Therefore the ampelographic descriptions are the first step towards to the identification and the full understanding of the agronomic and genetic structure of these minor varieties. These varieties were collected from 1914 to 2005 in all the geography of Balearic Islands and then introduced in the *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain), however, nowadays some of this varieties are not 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.

Therefore we examine the following objectives: (1) whether 27 vine accessions differ among themselves using morphological and genetical analysis methods, (2) whether the identification of the most influential descriptors and their relationships with the described varieties could help to make easier the field descriptions, (3) whether occasional climatic phenomena (hailstorm) conditioned agronomic, ampelographic descriptions and phenology and (4) whether the ampelographer’s experience and objectivity influences ampelographic descriptions, identifying the more difficult descriptors.

Material and Methods

Site description and climate data

The study was carried out at the *Vitis* Germplasm Bank (VGB) “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain; 40º31´N, 3º17´W, 610 m asl, semiarid Mediterranean climate). The climatic data were obtained from the meteorological station located inside the VGB. Average precipitation (mm) and temperature (ºC) were summarized monthly over the study period (January 2006-December 2007), since certain climatic parameters may interfere in ampelographic descriptions (Alleweldt and Dettweiler 1989). The 52-years mean monthly rainfall and temperature for this station were also included (period 1956-2007).

Plant material

The material described coincides with the varieties of *Vitis vinifera* L. introduced in the *Vitis* Germplasm Bank (VGB) “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain) from Balearic Islands (Table 1), except three accessions of Beba variety (O16, O17, O44), coming from Valencia and the variety Pampolat girat coming from Tarragona. Twenty seven different accessions planted in 2002 were described, all of which are grafted on Richter 110, simple cordon pruning, eight buds per vine and were adult plants. The planting density was 4808 vines ha^{-1} (0.80 m $\times$ 2.60 m).

Table 1. Plant material from the Balearic Islands located at the *Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain). Local names=names of the accession; Acc.: accession; Id.: code used in the analysis; Berry Colour: N=red, B=white, RS=rose; Use: W=wine, T=table

Local names	Acc.	Id.	Berry colour	Use	Bank code
Batista	E23	BAT.E23	N	W	ESP080-BGVCAM1109
Mateu	E39	BEB.E39	B	W	ESP080-BGVCAM0901
Corazón de Ángel	O16	BEB.O16	B	W	ESP080-BGVCAM0890
Valenci blanco	O17	BEB.O17	B	W	ESP080-BGVCAM1097
Grumiere blanco	O44	BEB.O44	B	W	ESP080-BGVCAM2187
Jaumes	E14	BEB.E14	B	W	ESP080-BGVCAM1543
Calop	E32	BEB.E32	B	W	ESP080-BGVCAM1119
Calop blanco	E43	BEB.E43	B	T	ESP080-BGVCAM2325
Beba negra, Calop negro, Calop negre	E18	BEN.E18	N	T, W	ESP080-BGVCAM1537
Calop rojo, Calop roig	E19	BER.E19	RS	W	ESP080-BGVCAM1538
Vinaté, Vinater	E11	BOB.E11	N	W	ESP080-BGVCAM1550
Boal	E21	BOB.E21	N	W	ESP080-BGVCAM1535
Callet	E26	CAL.E26	N	W	ESP080-BGVCAM1118
Eperó de Gall, Esperó de gall	E37	EPE.E37	N	W	ESP080-BGVCAM0893
Excursach, Escursach	E27	EXC.E27	N	W	ESP080-BGVCAM1131
Fogoneu	E24	FOG.E24	N	W	ESP080-BGVCAM1132
Mancés de Capdell	E31	GIR.E31	N	W	ESP080-BGVCAM1143
Giró	E36	GIR.E36	N	W	ESP080-BGVCAM1134
Gorgollasa, Gorgollassa	E25	GOR.E25	N	W	ESP080-BGVCAM1135
Mansés de tibbus, Mancés de Tibbus	E30	MES.E30	N	W	ESP080-BGVCAM1144
Manto Negro	E29	MTN.E29	N	W	ESP080-BGVCAM1145
Cabellis	E20	MTN.E20	N	W	ESP080-BGVCAM1536
Pampolat Girat	H24	PAM.H24	N	W	ESP080-BGVCAM1884
Pensal blanco, Moll, Prensal blanco	SCL	PEN.SCL	B	W	ESP080-BGVCAM2682
Massacamps	E38	QUI.E38	B	W	ESP080-BGVCAM0900
Quigat	E33	QUI.E33	B	W	ESP080-BGVCAM1159
Sabaté, Sabater	E35	SAB.E35	N	W	ESP080-BGVCAM1162

Ampelographic description

Ampelographic descriptions were carried out during two consecutive years (2006 and 2007; UPOV 2007). Additionally old descriptions developed in 1998 and 1999 (stored in VGB) were used as reference. The observations were made according to Office International de la Vigne et du Vin (OIV 1984) specifications, recording a total of 58 characters in the precise phenological state (including qualitative and quantitative characters; Table 2). During year 2006 descriptors recommended by OIV (1984) modified by Genres 081 were used (www.genres.de/vitis/vitis.htm), but in 2007 we used updates for the descriptors published, which are specified in the project Grapegen 06 (http://news.reseau-concept.net). Therefore, it was necessary to standardize descriptions of 1998-1999 and of 2006 according to 2007 descriptors. All varieties were described with a minimum of five plants (UPOV 2007). A minimum of 10 young shoot, adult leaves, shoots, bunches and woody shoot for each vine were sampled, except for berries which 30 belonging to the middle of ten representative bunches were took. All varieties were described by three ampelographers with different lack of experience, therefore, a minimum of 30 data for each of the descriptors were provided. At least two repetitions for each of the descriptions in each of the accessions were realized, selecting the modal value as a final description.

Table 2. List of the descriptors used (OIV 1984)

Parameters	Part plant	Descriptors
Qualitative parameters	Shoot tip	OIV-001, OIV-002, OIV-003, OIV-004
	Young leaf and shoot	OIV-006, OIV-007, OIV-008, OIV-015-2, OIV-016, OIV-051, OIV-053
	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
	Fenologhy	OIV-301, OIV-303
Quantitative parameters	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

Molecular characterisation: microsatellite analysis

DNA was isolated by extraction from young leaves using the DNeasy Plant kit (QIAGEN, California, USA). A set of 6 microsatellite loci proposed by the GENRES 081 Project (European *Vitis* Database, www.genres.de/vitis/vitis. htm) was analyzed in order to verify the varietal identity as described Martín et al. (2003) with minor modifications such as substitution of microsatellite *ssrVr*ZAG47 by VVMD27. PCR amplifications were analyzed by an ABI 3130 Genetic Analyzer (Applied Biosystem, 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, Granache, Merlot and Mourvèdre; This et al. 2004).

Agronomic and must variables

The following agronomic variables were recorded: yield variables (number of bunches per shoot, number of bunches per vine (on each of the vines in harvest), number of woody shoots per vine, yield (kilograms grape per vine) and woody shoot weight (on each of the vines in pruning)); variables of bunch and berries 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 recorded must variables were: must yield (%) (OIV-233), following the methodology described by Santiago et al. (2008): the supernatant obtained for this measurement was used to determinate the probable alcohol content (%VOL; OIV-505) that was first measured in ºBrix, using a hand held Brix refractomer (PR-101, Palette, ATAGO); then a conversion Table was used to determine the probable alcohol content (%VOL); pH (OIV-508) was measured using a pH meter (Crison Micro GLP21, Alella, Barcelona, Spain) and total acidity (g L^{-1} Tartaric acid; OIV-506); where berries from the central part of each representative bunch were selected, crushed and the total acidity of the must calculated described by Santiago et al. (2008).

Phenology

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

Data analysis

The climate data were analyzed using descriptive statistics, whereas the agronomic and ampelographic description dataset was 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 which that discriminating power. Hierarchical clusters were 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). As a characterization was developed during two consecutive years different dendrograms were generated for each studied 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 (Alleweld and Dettweiler 1989; Agresti 2002; García-Muñoz et al. 2005). The correlations between the descriptions of the years 2006 and 2007 as well as the 1998-1999 reference descriptions available in VGB were analysed using two-way Mantel tests (Legendre and Legendre 1998). This analysis has been used also to test if a spot phenomenon such as a hailstorm generated differences on the descriptions.

Univariate methods comprised Student-t test for paired data to evaluate the existence of significant differences between the quantitative descriptors measured in the years 2006 and 2007 over the same individuals (Crawley 2007). Quantitative descriptors (that are qualitative characters according to OIV) were those of agronomic interest related to bunch, must as well as berry weight, number of bunches per shoot, number of bunches per vine, yield (kg 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 and number of bunches per vine, number of vine shoots per vine) were transformed using log-transformation (Crawley 2007).

To test the existence of discrepancies between the qualitative ampelographic descriptions considering the experience of the observer, 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 only 35 characters were used excluding the characters related to length and width berry (OIV-220, OIV-221), shoot attitude (OIV-006), phenology, quantitative characters and those characters that were stables in all accessions (OIV-001, OIV-016, OIV-151, OIV-230, OIV-231 and OIV-241). For each of the 35 descriptors a contingency table was made relating the experience of observer and controls versus the number of occurrences presented at each level of expression for each one of the OIV descriptors. Over 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 if in doubt the ampelographic photography descriptions results were used. Finally, binomials test for two proportions were used to analyse the differences between the presence/absence of teeth on the petiole sinus, number of wings per bunch and fenology for the years 2006 and 2007 (Crawley 2007). The proportions were calculated for each expression level of each OIV descriptors for each year.

The statistical analysis of similarity, hierarchial clusters, CA and Mantel test were performed with the software Numerical Taxonomy System (NTSYS v.2.1; Rohlf 2000). The remaining analyses (generalized linear models, Student's t 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

Climatic data

The temperature and precipitation observed during the period 2006 and 2007 was similar to 52-years series average, although in year 2007 the rainfalls showed an atypical distribution (Figure 1). While, the rainiest period of 2006 was winter, in 2007 the period with the most rain was spring, caused due to the hailstorm that took place on May 20th in which 43 mm were collected, which represents 44% of that month’s rain. This hailstorm phenomenon in the area being studied occurs with a frequency of once every 5 years.

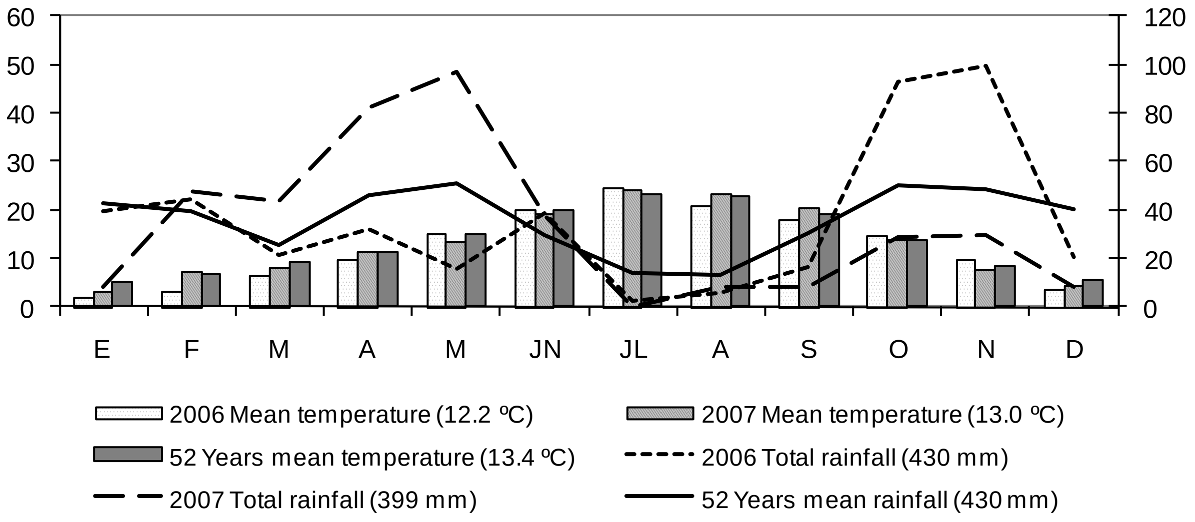

Figure 1. Monthly mean precipitation (mm) and temperature (ºC) for the years 2006, 2007 and 52-years period, based on "Finca El Encín" Meteorological Station (40º31´N, 3º17´W, 610 m asl, Alcalá de Henares, Madrid, Spain)

Differences among varieties using morphological and genetic approaches

The description carried out in the year 2006 showed a high degree of similarity between the repetitions of one single accession (Figure 2). The lowest rate of similarity was found in Batista variety and showed a rate of 0.85 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) to 0.99 (Manto Negro; E20 accession). At a similarity level of 0.75, four groups can be distinguished (Figure 2). The first one, with a 0.75 similarity level among individuals, was made up by the red Beba Negra and Fogoneu varieties. The second group, with a high similarity level among groups of 0.90, is formed by three red varieties, Excursach, Callet and Manto Negro, this varieties generally have very low-intensity colouration of the prostrate hairs of the tip and no or very low presence of 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 following group, with a similarity between its individuals of 0.81, is formed by a rose variety (Beba roja) and three white varieties, Pensal Blanca, Beba blanca (the similarity between this variety's seven accessions studied is 0.91) and Quigat. The last group, with a similarity between individuals of 0.78, is formed by the red varieties Eperó de gall, Bobal, Gorgollasa, Pampolat girat, Giró, Mansés de Tibbus and Sabaté, the similarity between the repetitions for the same accession was always more 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 leaf and the young leaf's main veins. The Batista variety is separated from all the varieties, although it would be much closer to last two groups, but with lower similarity level (0.61).

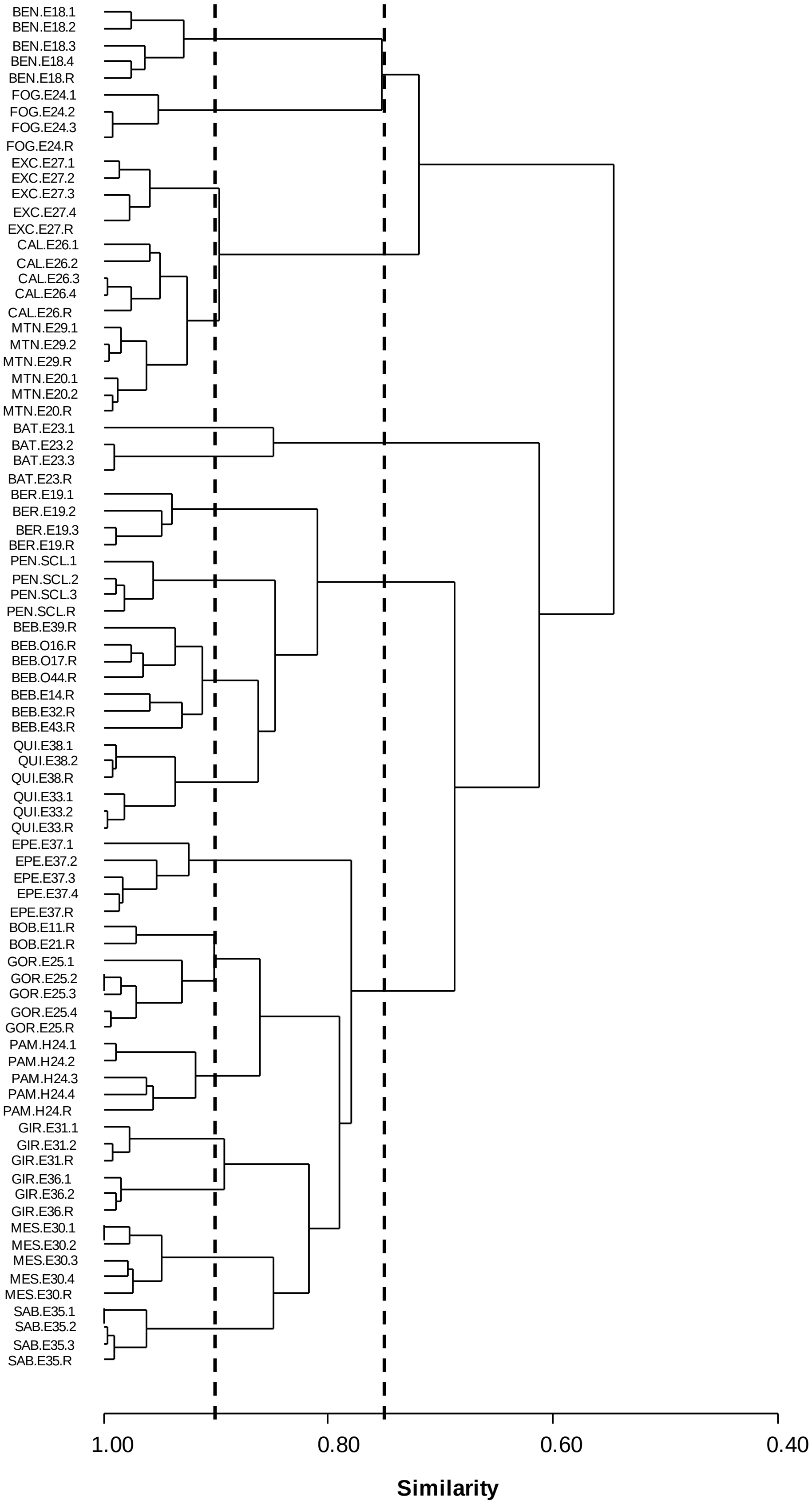

Figure 2. Cluster dendrogram based on morphological relations among Balearic Islands vine varieties described in 2006 (*Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain), using UPGMA method and correlation coefficient. Codes in Tab 1. The number after accessions indicates repetitions and R is the modal result of the descriptions realized, whereas lines indicate 0.75 and 0.90 similarity cuts

Regarding the descriptions of 2007, the rate of similarity between the repetitions of one single accession is very similar to the observed in 2006 (Figure 3). Again, Batista variety 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 Blanca and Pampolat girat varieties to 0.98 for Callet, Beba roja and Sabaté. The grouping of varieties in the year 2007 agree largely with the grouping obtained with the 2006 data, although with certain differences that could be due to the damage caused by the hailstorm (Figures 2, 3).

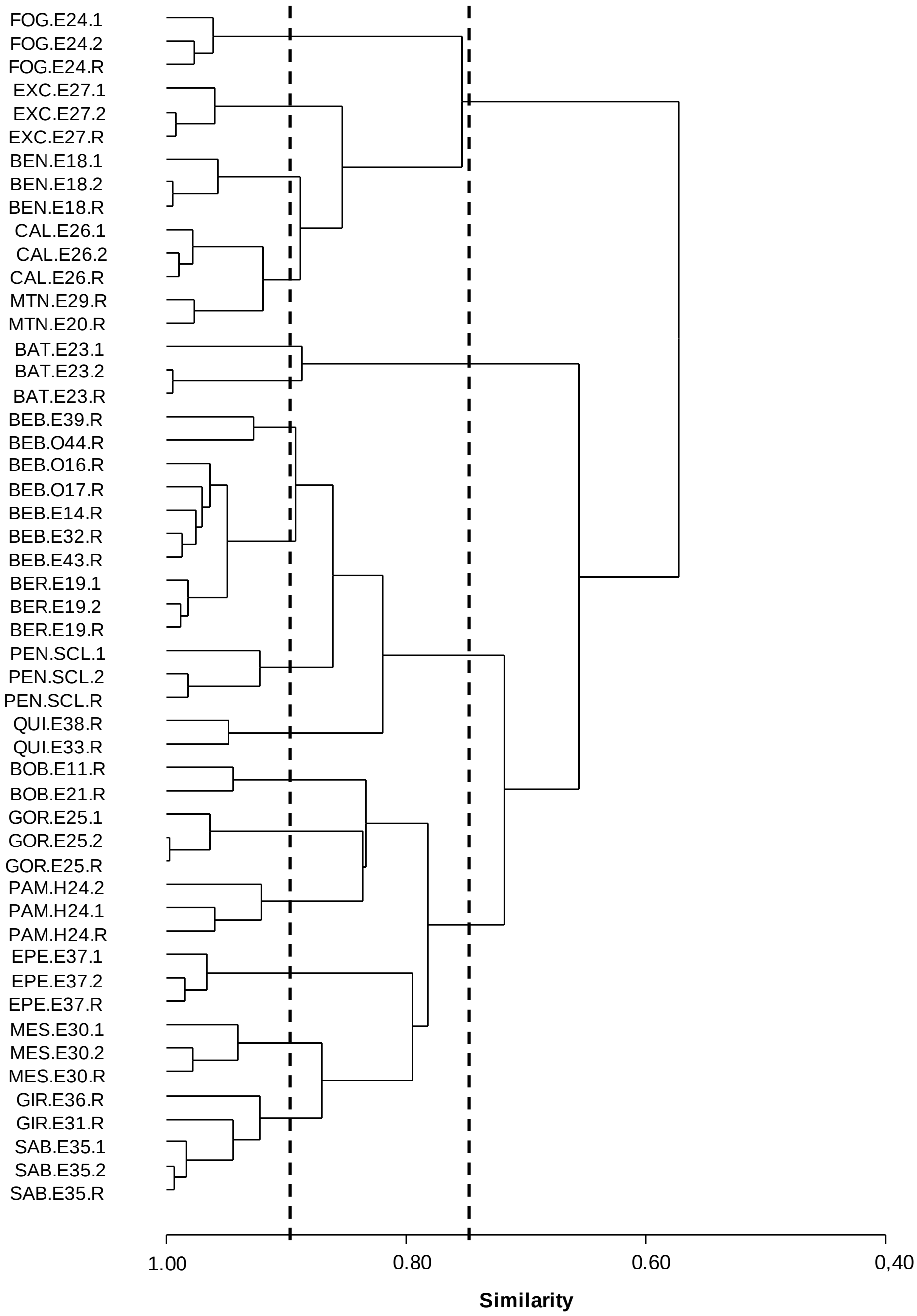

Figure 3. Cluster dendrogram based on morphological relations among Balearic Islands vine varieties described in 2007 (*Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain), using UPGMA method and correlation coefficient. Codes in Tab 1. The number after accessions indicates repetitions and R is the modal result of the descriptions realized, whereas lines indicate 0.75 and 0.90 similarity cuts

The Mantel test showed a good correlation among all the descriptions. The 2006 and 2007 descriptions showed a correlation rate of 0.87 and 0.74 with the 2006 and 1998 and 1999 (ampelographical reference descriptions). The best correlations between 2006 and 2007 for each one of the described plant parts were obtained in shoot tip (r=0.92), and the worst correlation 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.

Regarding the genetic characterization, the six SSR markers selected grouped the 27 samples analyzed in 16 different genotypes. The white accessions E39, O16, O17, O44, E14, E32 and E43 showed the same microsatellite profile as Beba roja (E18). The two accessions E11 and E21 shared the same microsatellite profiles, similar results were showed by E31 and E36, E20-E29 and E33-E38, respectively (Table 3). These results are in agreed with the ampelographyc descriptions.

Table 3. Genetic profiles at six microsatellite loci (in pb) of the 27 vine accession. Local names=names of the accession; Id.: code used in the analysis; Main name: name of the variety. Cabernet-Sauvignon, Chardonnay, Granache, Merlot and Mourvèdre varieties were used as reference (Ref)

Local names	Id.	Main name	VVMD7		VVS2		VVMD5		VVMD27		ZAG62		ZAG79	
Batista	BAT.E23	Batista	237	251	141	151	229	235	179	189	188	200	245	251
Mateu	BEB.E39	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Corazón de Ángel	BEB.O16	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Valenci blanco	BEB.O17	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Grumiere blanco	BEB.O44	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Jaumes	BEB.E14	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Calop	BEB.E32	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Calop blanco	BEB.E43	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Beba negra, Calop negro, Calop negre	BEN.E18	Valenci Tinto	237	241	135	141	225	233	181	189	188	196	243	247
Calop rojo, Calop roig	BER.E19	Beba	241	247	133	141	233	237	181	189	188	204	243	247
Vinaté, Vinater	BOB.E11	Bobal	237	241	144	146	225	231	181	189	188	188	243	247
Boal	BOB.E21	Bobal	237	241	144	146	225	231	181	189	188	188	243	247
Callet	CAL.E26	Callet	237	247	131	141	233	237	181	189	188	196	243	247
Eperó de Gall, Esperó de gall	EPE.E37	Eperó de gall	241	247	131	144	223	231	179	181	188	204	243	257
Excursach, Escursach	EXC.E27	Escursach	237	237	141	144	223	237	181	181	188	196	247	251
Fogoneu	FOG.E24	Fogoneu	237	245	131	144	233	237	179	181	196	204	247	251
Mancés de Capdell	GIR.E31	Giró	245	247	131	131	223	233	179	181	204	204	247	247
Giró	GIR.E36	Giró	245	247	131	131	223	233	179	181	204	204	247	247
Gorgollasa, Gorgollassa	GOR.E25	Gorgollassa	237	247	141	151	219	237	179	194	188	204	257	261
Mansés de tibbus, Mancés de Tibbus	MES.E30	Manses de tibbus	241	247	131	151	219	223	181	181	188	204	243	243
Manto Negro	MTN.E29	Manto Negro	237	241	131	144	231	233	181	194	186	188	247	257
Cabellis	MTN.E20	Manto Negro	237	241	131	144	231	233	181	194	186	188	247	257
Pampolat Girat	PAM.H24	Pampolat girat	241	247	131	141	233	237	181	183	188	204	247	259
Pensal blanco, Moll, Prensal blanco	PEN.SCL	Pensal Blanca	237	241	135	141	231	231	179	194	188	196	249	257
Massacamps	QUI.E38	Quigat	241	247	144	151	229	231	181	181	186	188	247	261
Quigat	QUI.E33	Quigat	241	247	144	151	229	231	181	181	186	188	247	261
Sabaté, Sabater	SAB.E35	Sabaté	237	241	131	131	225	231	185	194	188	196	237	257

Local names	Id.	Main name	VVMD7		VVS2		VVMD5		VVMD27		ZAG62		ZAG79	
Cabernet sauvignon	Ref	Cabernet-Sauvignon	237	237	137	151	229	237	175	189	188	194	247	247
Chardonnay	Ref	Chardonnay	237	241	135	141	231	235	181	189	188	196	243	245
Grenache	Ref	Grenache	237	241	135	144	223	237	194	194	188	188	257	257
Merlot	Ref	Merlot	237	245	137	151	223	233	189	191	194	194	259	259
Mourvèdre	Ref	Mourvèdre	247	247	131	151	223	237	179	189	188	204	251	261

The most influential descriptors

The CA results are shown separately for each of the years studied. The OIV-001, OIV-016, OIV-151, OIV-230, OIV-231 and OIV-241 descriptors were stable in 2006 and 2007, as well as the OIV-081-2 descriptors in the year 2007. The first two axes of the correspondence analysis in the description of the year 2006 explained 52% of the variance (Figure 4). The first axis absorbed 30% of the variance being OIV-225 and OIV-087 the most distinguishing characteristics. The second axis explained 22% and was related with OIV-084, OIV-053 and OIV-004 descriptors.

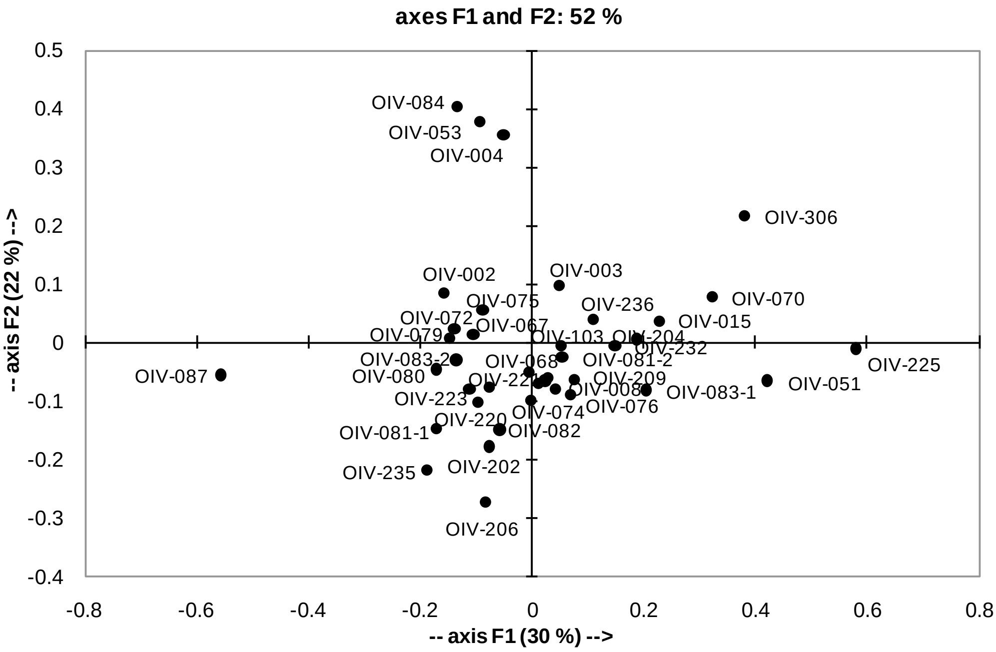

Figure 4. CA ordination diagrams of the first two axes of the OIV characters for the vine varieties from the Balearic Islands preserved in *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain): descriptions of 2006

The CA of 2007 showed that variance explained by the first two axes was 49% (Figure 5). Axis 1 explained 28% of the variance and was related with OIV-225, OIV-084, OIV-053 and OIV-004 descriptors. Axis 2 explained 21% of the variance and it was influenced by the OIV-202 and OIV-070 descriptors. These results were largely in line with the grouping of the varieties in the 2006 and 2007 dendrograms.

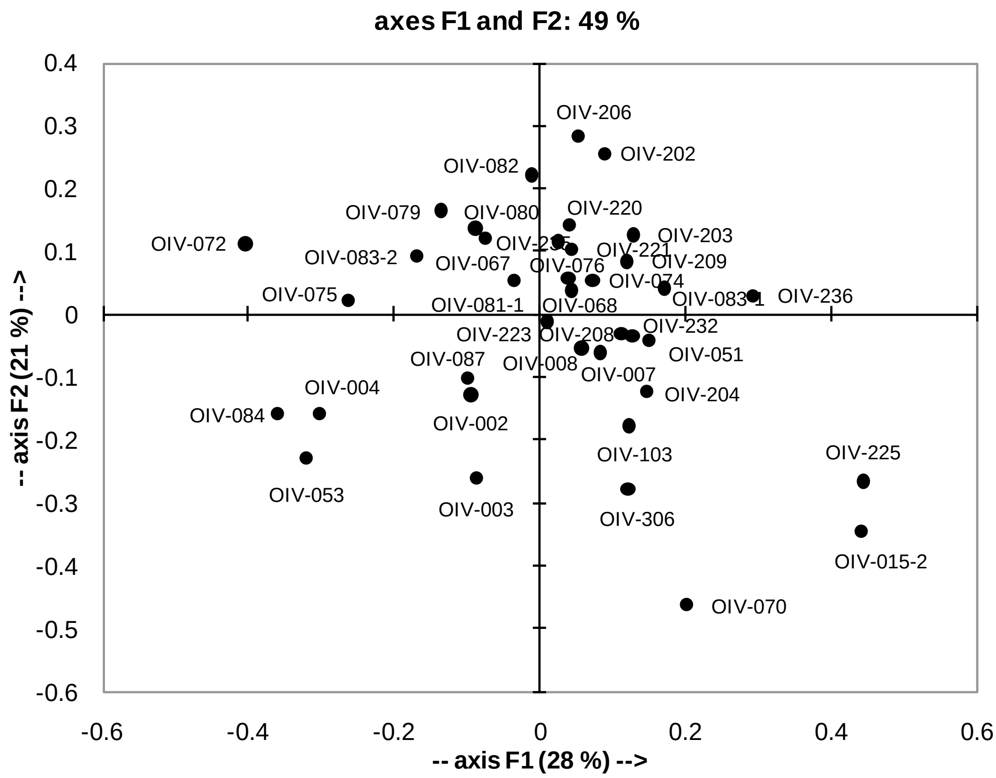

Figure 5. CA ordination diagrams of the first two axes of the OIV characters for the vine varieties from the Balearic Islands preserved in *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain): descriptions of 2007

Variations on ampelographic descriptions, agronomic and phenological characters

The differences between 2006 and 2007 ampelographic quantitative dataset were statistically significant (*z*-value=2.461, *p*-value=0.014) in the presence of teeth in the petiole sinus of adult leaf, being more frequent in 2006 that in 2007 (63% in comparison with 30%). With respect to the number of the bunch’s wings, there were also statistically significant differences. In the year 2006, 7% of bunches had no wings, while in 2007, there was no bunch without wings (*z*-value=2.836, *p*-value=0.004), whereas in 2007 there were 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 agronomical parameters showed statistically significant differences (Table 4). The number of bunches per shoot in 2006 was almost double that of 2007 (1.10 compared with 0.65), the vines had an average of 8 bunches in the year 2006, while in 2007 this was reduced to 6. With regards to grape production it was greater in 2006 than in 2007 and it dropped from 1.64 kg vine^{-1} in 2006 to 0.87 kg vine^{-1} in 2007. However, the number of vine woody shoots per vine and the weight of the vine shoot also showed statistically significant differences, however in contrast to what happened with the rest of the parameters, it was greater in 2007 (from 7 to 9 woody shoots per vine and from 34 to 45 g per woody shoot, respectively). Regarding the quantitative parameters, relating to the bunch and the berry, showed significant differences in two of them (Table 4), the length of the stalk 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). The parameters related with the characterization of the must (Table 4) showed significant changes in pH and total acidity, being 2007 samples more acidic. Therefore, they had a lower pH than the 2006 samples.

Phenology showed significant differences with regards to the duration of the vegetative period only from the time when the hailstorm occurred at which all the plants had the same stage: flower buds separated (stage H). Until then, for the two years being studied, 18 days had gone by since the buds had burst (stage C). The differences were marked from that moment until ripeness, and there were significant differences between the two years studied (t-value=4.491, p-value<0.001), being the duration of vegetative period longer in 2007 (144 days) than in 2006 (136 days). In 2007 all the varieties had a longer period for phenological stages, since the separated flower buds to ripeness (t-values>1.706, p-values<0.05).

Table 4. Mean values (± standard error) of qualitative agronomic and enological parameters of the vine varieties from Balearic Islands located at *Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain). *P-values* and degrees of freedom (*df*) derived from a paired *t*-test are presented

	Variables	2006	2007	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 grape 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	0.100	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	25.6±1.15	1416	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	6753	<0.001	24
	Total acidity (g L^{-1} TH_2)	4.72±0.21	5.66±0.20	3839	<0.001	24

Ampelographer's experience and objectivity influenced qualitative ampelographic descriptions

For the year 2006, out of the 35 characters studied, only 10 (28.6% of the total) not showed independence between the ampelographer and the description (Table 5), therefore observer's experience had an influence over these characters descriptions. 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). However, for the year 2007, the observer's experience had an influence over the descriptions of 4 characters (11.4% of the total; Table 5), 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 (OIV-235).

Table 5. OIV characters which showed significant differences between the ampelographers and control descriptions in 2006 and 2007 years, for vine varieties from Balearic Islands located at *Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain). No significant differences (ns)

	2006	**2007**
Descriptor	***p* (Chi)**	***p* (Chi)**
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	0.020	ns
OIV-232	<0.010	0.002
OIV-235	<0.010	<0.001

Discussion

Ampelographic descriptions and molecular analyses have proved to be very useful tools when describing *Vitis vinifera* accessions from Balearic Islands. In this study ampelography has been used as a preliminary technique for the clarification of vegetable material, and the results were later confirmed by microsatellite analysis. Although the varieties showed a large phenotipical similarity, they had certain characteristics (OIV-225, OIV-084, OIV-053 and OIV-004) which are key 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 the vine shoots, bunches and agronomic characteristics, as well as the composition of the berry (Calo et al. 1996). The experience of the people who make the descriptions also has influence on the ampelographic description. In fact, the subjectivity of certain characteristics, increased by the ampelographer's lack of experience, is a factor that complicates the correct description of the plant material (Ortiz et al. 2004).

Differences among varieties using morphological and genetic analysis methods

The morphological grouping of the varieties for which several accessions were studied, as is the case of Beba blanca, Bobal, Giró, Manto Negro and Quigat showed similarity rates of over 0.90 in the two studied years. This rate is the minimum for verifying homonyms and for clone selection processes (Cervera et al. 2002). The slight differences could be related with the variability typical of the plant material (Bessis et al. 2007), the polyclonal origin of the vine populations (Kozjak et al.

2003), changes in the virus load, epigenetic differences, somatic mutations or several combinations of these effects (González-Techera et al. 2004). Genetic analyses confirm this hypothesis, since these accessions showed the same micosatellite profiles. On the other hand, Callet and Manto Negro varieties are 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 pointed towards a parentage relationship between them (Hinrichsen et al. 2001). This hypothesis could be feasible, since due to their particular geographic location (both are only found in the Balearic Islands), could be the result of natural crosses involving the same varieties. 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). On the other hand, Batista seems to be different from the rest of the varieties that could be indicating a different genetic origin when compared with the rest of the varieties studied from the Balearic Islands.

It is remarkable the case of Beba blanca and Beba roja varieties. They have similarity levels of between 0.81 and 0.90 in the morphological analyses in 2006 and 2007, respectively. According to Martínez de Toda and Sancha (1997) with similarity rates below 0.85, the varieties could be considered as different varieties. Since they have identical genotypes, but clear morphological differences only referred to the colour of the berry (one is white and the other is rose) that could be explained by the incidence of somatic mutations and therefore should be considered as different cultivars (Laiadi et al. 2009).

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 and red varieties and one rose variety were studied, the density of prostrated hairs between the main veins of the adult leaf's lower side (OIV-084; Martínez de Toda and Sancha 1997) and the density of the hairs between the main veins of the young leaf (OIV-053; Allewelt and Detweiler 1989; Martínez de Toda and Sancha 1997), being the most discriminating characteristics and with a strong influence on the grouping of the varieties studied.

Variations on ampelographic descriptions, agronomic and phenological characters

The hailstorm phenomenon seems to be the cause of the great influence on the agronomic characterization parameters, increasing the rainfall in flowering and the damage on the vegetation that greatly affected the material to be described, however the temperature are largely in line between 2006 and 2007 data. The parts of the vine most affected by the hailstorm were the young shoot and the bunch, however the part least affected turned out to be the shoot tip.

The leaves showed differences mainly due to the presence of teeth in the petiole sinus. The characteristics of the presence of teeth were proved to be stable and objective (Ortiz et al. 2004) and, according to the results of this work, they are easily identifiable irrespective of ampelographers. Therefore, the statistically significant difference found between the two years of study could be blamed on the occasional climatic phenomena. Since the hailstorm strongly reduced the length of the young shoots, when it came to selecting the material to be described, 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 could change some characters of the bunch like the number of bunch wings, that in 2007 increased the number of bunches with 2 wings and dropped the number of bunches with 3 wings. This may have been due to either (a) the hailstorm on damaging certain parts of the bunch increased the visibility of the wings, or (b) because the bunch in its initial stage is capable to develop secondary growths as occurs in the 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, however the total length of the bunch was similar in 2006 and 2007, on the other hand, the length of the stalk varied a great deal, as shown by Theiler and Coombe (1985) in occasional processes of losses of flowers in the flower head, the growth of the stalk of the bunch stops immediately.

While the variety's ampelographic characteristics 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). Basically on the yield, as a result of the hailstorm there was loss of a large number of flower heads like was described by Reynier (2005) and caused by both the reduction in the number of bunches per vine and the number of berries per bunch, causing the drastically dropping on yield (kg $vine^{-1}$) during 2007, 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 the weight of the berry like in the study 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 was showing by Reynier (2005). With regards to the parameters of the must, 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 like was described by Jones and Davis (2000). In this study, acidity and pH were extremely influenced by the environment, similar results were found by Prenesti et al. (2004). For the years when there is a delay in phenology, these are years in which the levels of acidity increased as Jones and Davis described in 2000. The same occurred for the pH data, since these two variables were negatively related following the results of Zamboni et al. (1997).

Ampelographer's experience and objectivity influenced qualitative ampelographic descriptions

All the more discriminating characteristics according to Dettweiler (1993) do not change with the environment. They are also stable and objective (Ortiz et al. 2004) and, in this study, they were all established as characteristics about which no differences were found among the 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 check. With regards to the characteristics about which there do exist differences among the ampelographers, it should be pointed out that, in the second year of the study, the number of descriptors about which there were differences dropped considerably. This supports the idea of Ortiz et al. (2004), which emphasized that the ampelographers' experience is important, although the characteristics that define the juiciness of the flesh (OIV-232) and the firmness of the flesh (OIV-235) are proved to be variable in the two years of study and could be considered subjective. They are also characteristics that can vary with the period in which the description is made due to the change in the berry compositions throughout the ripening (Giovanelli and Brenna 2007). The characteristic of the colour of the upper side of the fourth leaf (OIV-051) even though it is considered by the OIV (1984) as a characteristic easy to assess, in this work it was not considered as such, because of the significant differences found when compared with the control check in the two years studied.

Conclusions

The morphological and molecular characterizations of 27 accessions recollected in Balearic Islands (Spain) were allowed to validate the ampelographic description method and grouped them in 17 vine varieties. It has been clear the importance of an accurate selection in the field of material to be evaluated, since it could to influence some characteristics, especially the presence of teeth on the adult leaf and the number of the bunch's wings. The "colour of the upper side of the fourth leaf" descriptor (OIV-051) should be excluded from the list of characteristics easy to assess issued by the OIV, since it showed significant differences with the control in both years of study.

The hailstorm influenced agronomic characterization: reduction in the weight of the bunch, decrease of the number of berries per bunch, and decrease in the yield (kg $vine^{-1}$) stands out. Regarding must quality, the meteorological accidents showed a high influence increasing acidity and decreasing pH. In addition, the hailstorm influenced the phenology 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. Over two consecutive years of study, the number of characteristics in which the ampelographers's differences varied from the control check dropped considerably.

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), this fact could be favoured. In order to corroborate the possible genetic relationships, further analysis involving larger molecular markers are required. Finally, the genetic analysis shows Beba blanca as a possible somatic mutant from Beba roja.

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Chapter 4

de Maggiolo V. (1535) *Mapa del Mediterráneo conposuit hanc cartam in Janua de anno de 1535 die V february*

Grape varieties (*Vitis vinifera* L.) from the Balearic Islands: genetic characterization and relationship with Iberian Peninsula and Mediterranean Basin

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Abstract

Ampelographic descriptions, a set of 20 nuclear microsatellite loci (nuSSR), five chloroplast microsatellites (cSSR), as well as historical references have been used to identify 66 accessions of *Vitis vinifera* L. The plant material included major and minor varieties under risk of extinction, collected in the Balearic Islands, and now conserved in two germplasm repositories site in Spain.

The 66 samples analyzed corresponded to 32 different genotypes, several unique genotypes were found, three of them remaining unknown. Some synonyms and homonyms were found in the Mediterranean basin, highlighting that the dispersal of some varieties are related with historical human movements and migrations occurred in three several periods, (1) around VII century related to Islam expansion, (2) around XIII-XV centuries and (3) in the XIX century related to phylloxera crisis.

Some parentages were identified, being the cultivar Callet Cas Concos a key variety in several crosses, confirming the high value of unknown varieties for parentage analysis. Several grouping methods confirm the existence of two gene pools.

Key words: nuclear microsatellite, chlorotype, minor varieties, genetic structure, parentage analysis

Introduction

Grapevine prospection (*Vitis vinifera* L.) around the world are rescuing varieties under risk of extinction (Santana et al. 2008; Boursiquot et al. 2009). Rescued plants are preserved in grapevine collections to prevent genetic erosion (This et al. 2006). It is estimated that there are approximately 10,000 grapevine cultivars held in germplasm collections worldwide (Santana et al. 2010). As usual for old resources, adaptations or changes of names as well as the lack of knowledge of ampelography has caused the appearance of many homonyms and synonyms (Aradhya et al. 2003). Therefore, the vegetal material found among the new prospection has to be well identified.

In grapevine, the combination of ampelography and microsatellite markers allows the identification of cultivars facilitating the management of collections (Lopes et al. 1999). Microsatellites markers are a powerful tool to distinguish cultivars, clarifying synonyms and homonyms (Lopes et al. 1999; Sefc et al. 2000), and to establish genetic relationships (Boursiquot et al. 2009; Vargas et al. 2009). In the same way, chloroplast microsatellite markers are useful to define the direction of the parental crosses (Vargas et al. 2009), and to approach the geographic origin of the grapevine cultivars (Arroyo-García et al. 2006; Imazio et al. 2006) which is usually difficult to establish due to the high material exchange (This et al. 2006).

The Balearic Islands, located in the Western part of the Mediterranean basin, are composed by two of the 10 greatest islands in the Mediterranean Sea. The Balearic wines are well known in the world for their high quality since Roman times (Hidalgo 2002). More recently, wines made from local Balearic varieties have won several international competitions.

Another important feature of Balearic cultivars is that due to islands isolation, the current gene pool in the Balearic Islands likely results from plant material exchanges in ports around the Mediterranean basin and from natural crosses occurring on the islands (Prentice et al. 2003). Thus, they might be used to disentangle the grapevine movements around occidental Mediterranean basin.

The Balearic viticulture has changed over the years; first the powdery mildew, afterwards the phylloxera, and nowadays the homogenization of international wine market have reduced drastically the genetic pool. In fact, only four varieties (Callet, Manto Negro, Pensal Blanca and Fogoneu) out of 20 allowed in Quality Demarcations of the Balearic Islands are local varieties, the rest are internationally spread as Cabernet-Sauvignon, Tempranillo or Chardonnay. Consequently, the local cultivars are decreasing over the years (This et al. 2006; de Mattia et al. 2007). The loss of cultivars is dramatic in the Balearic Islands since some of the old local cultivars are only conserved in germplasm repositories. As a consequence, the origins and genetic relationships of the local cultivars from this area are virtually unknown.

The aim of this work was to characterize the local cultivars growing in the Balearic Islands, and specifically (i) to clarify cases of synonyms and homonyms of these cultivars with varieties cultivated in other countries; (ii) attempt to know the geographic movement and migration of these varieties, and finally (iii) to establish the genetic relationship between them. Microsatellite analysis, ampelographic descriptions and synthesis of historical references have been used for these purposes.

Material and Methods

Plant material

We analyzed 66 accessions of *Vitis vinifera* L. natives from the Balearic Islands (Spain) and conserved in two different repositories: 33 preserved at the *Vitis* Germplasm Bank (VGB) “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain) and 33 preserved in the Germplasm Collection of Palma de Mallorca (GCPM, Spain; Table 1). The GCPM accessions were collected in Mallorca Island between 2000 and 2007, whereas the VGB accessions were collected from 1914 to 2000 mainly in the Balearic Islands. However, based on antique bibliography, four accessions of Beba variety were collected from Levante area and Girona and Pampolat girat variety from Tarragona (Table 1).

Table 1. List of the 66 accessions analysed from the Balearic Islands (except * from Levante area, ** from Girona, *** from Tarragona). List sorted according to their SSR genotypes

G[1]	Local name	Sample location[2]	Berry color	Prime names[3] at VGB "Finca El Encín"	Prime names[3] at INRA GGR Domaine de Vassal
1	Argamusa	VGB	white	Argamusa	Argemusa
2	Batista	VGB	black	Batista	Canari
2	Batista	GCPM	black	Batista	Canari
2	Batista	GCPM	black	Batista	Canari
2	Batista	GCPM	black	Batista	Canari
3	Calop	VGB	white	Beba	Valenci blanco
3	Calop blanco	VGB	white	Beba	Valenci blanco
3	Corazón de Ángel *	VGB	white	Beba	Valenci blanco
3	Grumiere blanco *	VGB	white	Beba	Valenci blanco
3	Jaumes	VGB	white	Beba	Valenci blanco
3	Mateu **	VGB	white	Beba	Valenci blanco
3	Valenci blanco *	VGB	white	Beba	Valenci blanco
3	Calop rojo, Calop roig	VGB	rose	Beba rosa	–
4	Beba negra, Calop negro, Calop negre	VGB	black	Valenci Tinto	Valensi noir
5	Callet	VGB	black	Callet	Callet
5	Callet JB	GCPM	black	Callet	Callet
6	Eperó de Gall, Esperó de gall	VGB	black	Eperó de gall	–
7	Excursach, Escursach	VGB	black	Excursach	Murescu
8	Fernandella	VGB	black	Fernandella	–
9	Fogoneu	VGB	black	Fogoneu	Fogoneu
9	Fogoneu francés	VGB	black	Fogoneu	Fogoneu
10	Galmete	VGB	black	Mandón	Garro=Mando
10	Sabater	GCPM	black	Mandón	Garro=Mando
11	6	GCPM	black	Mancés de Capdell	Mancens=Giro de Baleares
11	Giró	VGB	black	Mancés de Capdell	Mancens=Giro de Baleares
11	Giró	GCPM	black	Mancés de Capdell	Mancens=Giro de Baleares
11	Mancés de Capdell	VGB	black	Mancés de Capdell	Mancens=Giro de Baleares
11	Puig Major	GCPM	black	Mancés de Capdell	Mancens=Giro de Baleares
12	Gorgollasa, Gorgollassa	VGB	black	Gorgollassa	Gargollasa
12	Gorgollassa	GCPM	black	Gorgollassa	Gargollasa
13	Mansés de tibbus, Mancés de Tibbus	VGB	black	Mansés de Tibbus	–
14	Cabellis	VGB	black	Manto Negro	Manto Negro
14	Cabellis	GCPM	black	Manto Negro	Manto Negro
14	Manto negro	VGB	black	Manto Negro	Manto Negro
14	Manto negro	GCPM	black	Manto Negro	Manto Negro
14	Manto negro	GCPM	black	Manto Negro	Manto Negro
14	Manto negro	GCPM	black	Manto Negro	Manto Negro
14	Manto negro	GCPM	black	Manto Negro	Manto Negro
15	Pampolat Girat***	VGB	black	Pampolat girat	Cruixen
16	Massacamps	VGB	white	Quigat	Quigat
16	Quigat	VGB	white	Quigat	Quigat
17	Sabaté, Sabater	VGB	black	Sabaté	Sabaté
18	Viñaté	VGB	white	Viñaté	–
18	Vinater blanc	GCPM	white	Viñaté	–
19	9	GCPM	black	Isabelle	Isabelle
20	Peu de rata	GCPM	white	Chasselas cioutat	Chasselas cioutat
21	Jaumillo	GCPM	white	Santa Magdalena	Tempranilla blanca
22	Manto negro	GCPM	black	Tinto Velasco	Tinto Velasco

G[1]	Local name	Sample location[2]	Berry color	Prime names[3] at	
				VGB "Finca El Encín"	INRA GGR Domaine de Vassal
22	Valent negre	GCPM	black	Tinto Velasco	Tinto Velasco
23	Callet Cas Concos	GCPM	black	Callet Cas Concos	–
23	Manto negro	GCPM	black	Callet Cas Concos	–
23	Planta A	GCPM	white	Callet Cas Concos	–
23	Planta B	GCPM	white	Callet Cas Concos	–
24	Batista	GCPM	black	Unknown 1	–
25	Callet blanc	GCPM	white	Valent Blanc	–
25	Valent blanc	GCPM	white	Valent Blanc	–
26	Gafarró	GCPM	black	Gafarró	–
27	Giró ros	VGB	white, black	Giró Ros	Giro sardo
27	Giró ros	GCPM	white, black	Giró Ros	Giro sardo
28	Manto negro	GCPM	black	Unknown 2	–
29	Moll	GCPM	white	Pensal Blanca	–
29	Pensal blanco, Moll, Prensal blanco	VGB	white	Pensal Blanca	–
30	Manto negro	GCPM	black	Unknown 3	–
31	Boal	VGB	black	Bobal	Bobal
31	Vinaté, Vinater	VGB	black	Bobal	Bobal
32	Unknow	GCPM	white	Macabeo	Macabeu

[1] Genotype (see Table 2)

[2] Sample location (Collection maintaining, VGB (*Vitis* Germplasm Bank "Finca El Encín" (Alcalá de Henares, Spain); GCPM (Germplasm Collection of Palma de Mallorca, Spain))

[3] Prime names at *Vitis* Germplasm Bank "Finca El Encín" (Alcalá de Henares, Spain) and INRA Grape Germplasm Repository of Domaine de Vassal (Marsellan-plage, France), – Variety non present in Vassal collection.

Microsatellite analysis

DNA was extracted from young leaves using the DNeasy Plant kit (QIAGEN, Germany). The set of 20 nuclear microsatellite loci (nuSSR) analyzed was chosen on the basis of their quality and distribution across the 19 chromosomes of the grapevine genome (Doligez et al. 2006) and was already used in previous studies (Boursiquot et al. 2009; Vargas et al. 2009; Riahi et al. 2010). They were amplified in two independent multiplex PCRs as previously described by Ibáñez et al. (2009). Five chloroplast microsatellites (cpSSRs) were also analyzed using a multiplex PCR performed according to Ibáñez et al. (2009). Chloroplast haplotypes were named according to Arroyo-García et al. (2006).

PCR fragments were separated in an AB 3130 automated sequencer and fragments were sized with GeneMapper 4.0 software using GeneScan-LIZ 500 as internal marker (Applied Biosystems, Foster City, CA, USA).

Data analysis

Identifications, synonyms and homonyms

Microsatellite profiles were compared using Excel software first with 1,698 distinct genotypes of VGB “Finca El Encín”, then with 1,626 different genotypes found in literature and finally with 2,323 distinct genotypes from the INRA Grape Germplasm Repository of Domaine de Vassal (Marsellan-plage, France, www1.montpellier.inra.fr/vassal). Allele sizes were first standardized to compare all datasets results using several international varieties as references (This et al. 2004).

Parentage analysis

Parentage analysis was carried out using FaMoz software (Gerber et al. 2000) adapted to grape (Di Vecchi Staraz et al. 2007) with a discrepancy of three loci. It was evaluated among the 1,698 distinct VGB “Finca El Encín” genotypes and 2,323 distinct Vassal genotypes previously characterized with the same 20 nuSSR markers. In order to clarify the direction of the parentage (mother *vs* father) and the putative geographic origin, the five cpSSRs microsatellites were also used.

Genetic structure

Standard measures of genetic variation including number of alleles per locus (Na), the observed (Ho) and expected heterozygosity (He) and the probability of identity (PI) were calculated using the software IDENTITY v. 1.0 (Wagner and Sefc 1999) on single genotypes. The discrimination power (D) was calculated according to Tessier et al. (1999).

Different clustering methods were applied to check the consistence of the results. A dendrogram including 41 cultivars (32 single Balearic genotypes and 9 putative parentages) based on 20 nuSSR markers was constructed to study genetic relationships using the neighbour-joining method (Saitou and Nei 1987) and Da genetic distance (Nei et al. 1983). One thousand bootstraps over loci were performed to assess significance of the tree topology. POPULATIONS software v. 1.2.30 (Langella 2002) was used to represent the genetic similarities between accessions. The dendrogram was displayed with Tree-View software (Page 1996).

Structure v. 2.1. (Pritchard et al. 2000) based on 19 nuSSRs was applied to identify the genetic clustering since this program only can be use for unlinked markers (Pritchard et al. 2000). Ten runs with a burn-in of 50,000 and a run length of 100,000 iterations were performed for a number of clusters ranging from K=1 to K=10, using the admixture model. The optimal number of genetic clusters, K, was chosen following the guidelines of Evanno et al. (2005). Each variety was assigned in one group only if they had more than 70% of the inferred ancestry (Santana et al. 2010). The difference between groups were performed using ARLEQUIN v 3.1 (http://cmpg.unibe.ch/software/arlequin3/) computing F_{ST} pairwise using 1,000 permutations. Those results were tested using FSTAT v. 2.9.3.2

(http://www2.unil.ch/popgen/softwares/fstat.htm). This software was also used for allelic richness (As) calculations. Then, we performed factorial correspondence analysis (FCA) using Genetix v 4.05.2 (Belkhir et al. 1996-2004).

Results

Identifications, synonyms and homonyms

Among the 33 accessions from VGB "Finca El Encín", 21 different genotypes were identified and confirmed in Vassal Collection (Table 1). Among the 33 accessions from the Palma Collection, 30 accessions were identified in VGB "Finca El Encín" and confirmed in Vassal Collection (Table 1). These accessions corresponded to 20 different genotypes (Table 2). Thus, the 66 accessions represented 32 different genotypes, since some genotypes are located in both collections (Table 2). Seventeen accessions corresponding to 11 different genotypes (34.4%) did not match with any known genotype in the consulted databases (1,698 genotypes from VGB "Finca El Encín", 2,323 genotypes from Vassal and 1,626 genotypes from literature). Ampelography, nuSSRs, and checking the ancient bibliography allowed us to assign a prime variety name for 63 out of 66 accessions, identifying synonyms and homonyms (Table 1); unidentified varieties were named Unknown 1, Unknown 2 and Unknown 3. The synonyms of Beba and Batista varieties were discovered based on the literature, and confirmed with ampelographic data from Vassal repository (Table 1). We found five synonyms between the two collections: Batista (Palma de Mallorca; Spain) from VGB "Finca El Encín" matched with Canari (Ariège, France) from Vassal; Excursach (Palma de Mallorca, Spain) with Murescu (Corsica, France); Mansés de Capedell (=Giró; Palma de Mallorca, Spain) with Mancens (Pyrénées-Orientales, France); Pampolat girat (Tarragona, Spain) with Cruixent (Corsica, France), and Giró Ros (Felanitx, Manacor; Spain) with Giro sardo (Sardinia, Italy). Different homonyms were found for Batista, Callet, Mandón, Manto Negro, Sabaté and Viñaté cultivars, some of them remained unknown (Table 1).

Table 2. Nuclear SSR data and chloroplast haplotypes of the studied accessions and putative parentages (*) analysed in the VGB "Finca El Encín"

Genotype	Prime Name used at VGB "Finca El Encín"	VMC1b11		VMC4f3-1		VVIb01		VVIh54		VVIn16		VVIn73		VVIp31		VVIp60		VVIq52		VVIv37		VVIv67	
1	Argamusa	189	189	187	187	290	294	167	169	153	153	263	263	176	190	318	318	88	88	161	165	352	364
2	Batista	167	183	173	206	288	294	165	169	151	153	263	263	180	188	322	322	82	88	163	163	364	375
3	Beba	185	189	187	189	290	294	165	167	151	153	256	263	190	192	318	322	82	84	161	163	366	372
4	Valenci Tinto	185	189	175	187	290	294	165	167	151	153	256	263	190	192	322	322	84	84	152	163	366	372
5	Callet	167	189	175	187	290	290	167	167	151	159	256	263	190	192	318	322	84	88	161	163	372	375
6	Eperó de Gall	189	189	179	187	290	290	167	169	153	159	263	263	176	180	318	322	88	88	163	171	364	372
7	Excursach	185	189	173	175	290	294	167	167	151	151	263	263	190	194	306	318	82	88	163	171	364	375
8	Fernandella	173	185	181	183	290	294	167	169	151	153	256	263	188	192	318	318	82	88	171	171	362	364
9	Fogoneu	189	189	175	206	290	294	167	167	151	159	263	263	190	190	306	322	88	88	161	163	362	375
10	Mandón	173	189	167	206	290	290	167	169	153	159	263	263	176	192	318	326	88	88	163	165	364	372
11	Mancés de Capdell	167	189	189	206	290	290	165	169	151	159	263	263	188	190	318	322	88	88	161	165	362	364
12	Gorgollassa	185	189	167	179	290	290	167	167	153	159	263	263	176	180	318	326	88	88	163	171	358	364
13	Mansés de Tibbus	167	189	167	189	290	294	169	169	151	153	263	263	188	190	318	322	88	88	161	165	364	366
14	Manto Negro	167	189	203	206	290	294	167	167	153	153	256	263	176	176	318	322	84	84	163	177	364	364
15	Pampolat girat	173	173	173	187	290	294	165	167	151	159	263	263	180	188	322	322	84	88	163	177	362	364
16	Quigat	189	194	179	187	290	307	165	167	153	159	263	267	180	190	306	326	84	88	163	165	372	375
17	Sabaté	169	189	203	206	290	294	167	177	151	153	263	263	176	192	318	318	82	84	163	177	358	364
18	Viñaté	167	185	167	187	290	290	140	169	151	153	256	263	176	180	322	322	84	84	163	171	364	368
19	Isabelle	171	177	173	173	294	296	169	169	151	157	263	263	176	180	321	322	82	82	158	171	348	364
20	Chasselas cioutat	173	175	173	179	290	294	165	169	159	159	263	263	182	194	318	322	84	88	152	163	362	364
21	Santa Magdalena	185	185	167	173	288	290	169	175	153	159	256	263	176	194	320	322	84	88	161	177	364	372
22	Tinto Velasco	173	185	187	206	290	290	165	167	151	151	263	263	184	190	322	322	88	88	158	158	358	375
23	Callet Cas Concos	167	189	187	206	290	290	165	167	151	153	256	256	176	192	318	322	82	84	163	171	364	372
24	Unknown 1	167	189	206	206	290	294	165	167	151	153	256	263	176	190	322	322	84	88	161	171	372	375
25	Valent Blanc	189	189	173	206	290	290	167	169	151	159	256	263	176	190	318	318	82	88	165	171	358	366
26	Gafarró	167	189	175	206	290	294	165	167	151	151	256	263	176	190	318	322	84	88	161	171	362	372
27	Giró Ros	167	189	206	206	290	290	167	167	151	151	256	263	176	188	318	322	82	88	171	177	358	366
28	Unknown 2	185	185	183	206	290	290	167	167	151	153	256	263	176	190	322	322	82	84	161	171	358	364
29	Pensal Blanca	185	189	187	203	290	294	169	177	151	153	263	263	176	190	318	326	88	88	161	177	352	366
30	Unknown 3	167	189	203	206	290	294	167	169	153	153	256	263	176	190	318	322	82	84	163	177	364	364
31	Bobal	185	189	173	183	290	294	167	169	151	153	263	263	176	186	326	326	82	84	158	163	358	362
32	Macabeo	185	185	179	187	290	294	167	167	153	153	263	263	176	196	318	326	84	88	158	161	372	375

Genotype	Prime Name used at VGB “Finca El Encín”	VMC1b11		VMC4f3-1		VVIb01		VVIh54		VVIn16		VVIn73		VVIp31		VVIp60		VVIq52		VVIv37		VVIv67	
*	Albaranzeuli bianco	167	189	187	206	290	290	167	169	151	153	263	263	176	188	318	322	84	88	163	177	366	366
*	Albillo Mayor	167	185	167	183	290	290	140	167	151	151	256	261	176	180	322	322	84	84	171	171	368	375
*	Aspiran	167	185	179	189	290	294	165	169	151	153	263	263	180	188	318	322	88	88	161	163	362	364
*	Brustiano faux	173	185	173	179	288	294	167	167	151	153	263	263	196	196	318	322	82	88	158	163	360	375
*	Planta Fina de Pedralba	185	185	167	167	290	290	167	169	153	153	263	263	176	190	318	322	88	88	161	177	358	372
*	Graciano	173	185	179	206	290	290	167	167	151	159	263	263	180	192	310	318	88	88	165	177	358	366
*	Hebén	185	189	167	187	290	290	167	169	153	153	263	263	176	190	322	326	84	88	161	163	364	372
*	Legiruela	185	185	173	173	288	294	167	175	153	159	256	256	176	194	322	322	84	88	161	177	364	364
*	Monastrell	173	189	179	179	290	290	167	167	153	159	263	263	180	192	318	322	88	88	165	171	358	364

Genotype	Prime Name used at VGB “Finca El Encín”	VVMD21		VVMD24		VVMD25		VVMD27		VVMD28		VVMD32		VVMD5		VVMD7		VVS2		Haplotype
1	Argamusa	243	249	208	212	239	239	181	194	260	260	250	270	223	231	237	237	131	135	A
2	Batista	249	255	208	212	247	253	179	189	250	260	250	270	229	235	237	251	141	151	A
3	Beba	249	255	208	210	253	253	181	189	246	260	254	270	233	237	241	247	133	141	A
4	Valenci Tinto	249	255	208	210	253	253	181	189	260	262	238	254	225	233	237	241	135	141	D
5	Callet	255	255	212	218	239	253	181	189	238	260	238	238	233	237	237	247	131	141	A
6	Eperó de Gall	243	249	208	210	239	239	179	181	260	260	238	270	223	231	241	247	131	144	A
7	Excursach	243	249	210	218	239	239	181	181	246	260	254	270	223	237	237	237	141	144	D
8	Fernandella	243	249	208	210	237	239	181	181	246	260	248	260	225	225	231	247	131	144	D
9	Fogoneu	243	255	218	218	239	239	179	181	238	246	238	270	233	237	237	245	131	144	D
10	Mandón	249	249	208	210	253	261	183	194	246	260	238	270	223	237	237	237	141	151	A
11	Mancés de Capdell	243	255	208	218	239	247	179	181	238	260	238	260	223	233	245	247	131	131	D
12	Gorgollassa	249	249	208	210	253	261	179	194	236	260	238	270	219	237	237	247	141	151	A
13	Mansés de Tibbus	243	243	218	218	239	261	181	181	260	260	260	270	219	223	241	247	131	151	A
14	Manto Negro	243	255	210	218	239	239	181	194	246	260	254	270	231	233	237	241	131	144	A
15	Pampolat girat	249	249	212	216	253	253	181	183	246	246	254	254	233	237	241	247	131	141	A
16	Quigat	249	249	208	210	239	253	181	181	236	260	238	254	229	231	241	247	144	151	A
17	Sabaté	243	255	212	218	239	239	185	194	246	260	250	270	225	231	237	241	131	131	D
18	Viñaté	243	253	208	214	239	239	183	194	260	260	254	270	229	231	237	241	144	144	A
19	Isabelle	249	249	208	208	239	247	179	183	228	238	246	270	235	235	233	247	121	151	B
20	Chasselas cioutat	249	265	208	212	239	253	185	189	220	270	238	238	225	233	237	245	131	141	B
21	Santa Magdalena	253	255	208	210	237	239	185	194	236	260	250	270	225	235	237	245	131	144	A
22	Tinto Velasco	255	255	208	214	237	237	179	185	250	262	250	250	229	235	231	251	131	131	A
23	Callet Cas Concos	243	255	210	212	239	253	181	189	260	260	238	254	231	233	237	247	141	144	A

Genotype	Prime Name used at VGB “Finca El Encín”	VVMD21		VVMD24		VVMD25		VVMD27		VVMD28		VVMD32		VVMD5		VVMD7		VVS2		Haplotype
24	Unknown 1	243	255	212	218	239	239	181	181	246	260	238	270	233	237	245	247	144	144	A
25	Valent Blanc	249	249	208	212	253	253	179	181	246	260	270	270	223	237	237	237	141	141	A
26	Gafarró	255	255	212	218	239	253	179	181	246	260	238	238	231	237	237	247	131	141	A
27	Giró Ros	243	249	208	212	239	253	179	181	246	260	238	270	231	237	237	237	141	144	D
28	Unknown 2	243	249	208	218	237	239	181	183	238	260	270	270	225	237	237	241	131	144	D
29	Pensal Blanca	243	255	208	212	239	253	179	194	260	262	250	254	231	231	237	241	135	141	A
30	Unknown 3	243	255	208	212	239	253	181	194	246	260	254	270	231	233	237	241	139	141	†
31	Bobal	243	243	208	210	239	265	181	189	236	262	248	270	225	231	237	241	144	146	A
32	Macabeo	243	253	208	210	237	239	189	194	238	260	248	254	233	233	237	237	131	144	A
*	Albaranzeuli bianco	243	249	208	208	239	253	179	194	236	246	238	270	231	233	237	241	141	144	A
*	Albillo Mayor	247	253	214	218	239	253	183	194	236	260	250	270	229	233	237	251	141	144	A
*	Aspiran	249	255	208	212	239	247	179	181	230	260	238	260	223	223	245	247	131	131	D
*	Brustiano faux	249	253	208	208	237	239	181	189	238	260	248	254	233	233	237	237	131	144	A
*	Planta Fina de Pedralba	249	253	210	218	239	253	179	194	250	260	248	270	225	237	237	241	141	144	A
*	Graciano	249	253	208	208	261	269	179	183	246	260	238	254	223	235	237	237	137	151	A
*	Hebén	243	249	208	210	239	253	181	194	236	260	254	270	231	237	237	241	141	144	A
*	Legiruela	249	255	208	208	237	247	185	189	236	246	250	270	225	235	231	245	131	155	A
*	Monastrell	243	249	208	218	239	261	179	189	246	260	238	254	223	237	247	247	131	151	A

† missing data

Parentage analysis

The 32 single genotypes were analyzed for possible parent-offspring relationships. We obtained 13 putative parentages (i.e. father-mother-son; Table 3), six of them with full compatibility for all nuclear microsatellites studied and for the chlorotype of both parents (Callet, Eperó de Gall, Gafarró, Manto Negro, Unknown 1 and Viñaté). Five oriented crosses were revealed. In the case of pedigrees not resolved with chlorotype data, the female status of Hebén and Excursach varieties determined the mother for six other pedigrees, two of them showed also a bootstrap value above 70% in the dendrogram. Actually, two lineages (kin-groups) were pointed out, one related with Callet Can Concos variety and one with Hebén variety (French synonym: Gibi; Figure 1).

Table 3. Thirteen putative parentages. LOD scores obtained using FaMoz software. Capital letters in brackets correspond to the haplotype defined by Arroyo-García et al. (2006, Table 2). The female parentage might be identified when both parentages showed the same haplotype and the variety is female

Progeny	Parent 1	Parent 2	Consistent loci	LOD score
Callet (A)	Callet Cas Concos (A)	Fogoneu (D)	20/20	28.31
Callet Cas Concos (A)	Beba (A)	Giró Ros (D)	19/20	27.50
Eperó de Gall (A)	Hebén (A)*	Monastrell (A)	20/20	23.65
Fogoneu (D)	Excursach (D)*	Mansés de Capdell (D)	19/20	27.62
Gafarró (A)	Callet Cas Concos (A)	Fogoneu (D)	20/20	23.77
Giró Ros (D)	Valent Blanc (A)	Albaranzeuli bianco (A)	20/20	25.87
Gorgollassa (A)	Hebén (A)*	Monastrell (A)	19/20	23.20
Macabeo (A)	Hebén (A)*	Brustiano faux (A)	19/20	26.42
Mandón (A)	Hebén (A)*	Graciano (A)	19/20	25.04
Manto Negro (A)	Callet Cas Concos (A)	Sabaté (D)	20/20	46.82
Santa Magdalena (A)	Planta fina de Pedralba (A)	Legiruela (A)	19/20	24.58
Unknown 1 (A)	Callet Cas Concos (A)	Fogoneu (D)	20/20	42.67
Viñaté (A)	Hebén (A)*	Albillo Mayor (A)	20/20	47.36

* Female variety

Lineage 1

Lineage 2

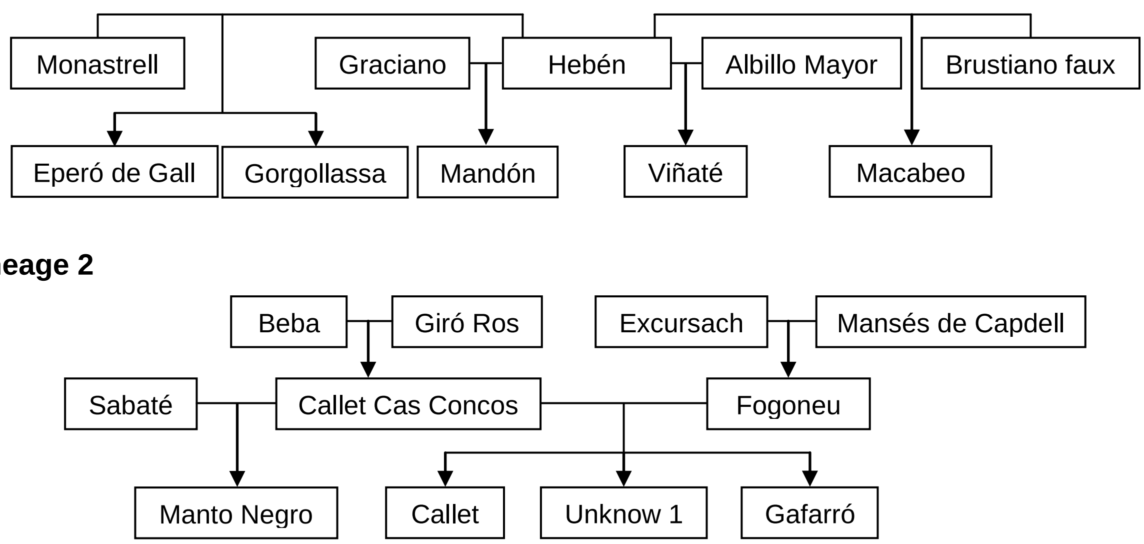

Figure 1. Lineages and parentages proposed for the Balearic Islands varieties using 20SSRs. Only the most reliable parentages are shown

Genetic structure

All microsatellite markers were polymorphic with a number of alleles per locus ranging from 3 to 10 (Table 4). A total of 136 alleles and a mean of 6.8 alleles per locus were found. The mean discrimination power per locus was 80.3%, although VMC4f3-1 showed the highest discrimination power (D=93.6%) and VVIn73 the weakest (D=11.9%; Table 4). The cumulative probability to find different individuals with the same profile for each of the 20 nuSSR was 2.322 x 10^{-19}. In this study, haplotype A (67.7%), B (6.5%) and D (25.8%) were found (Table 2).

Table 4. Genetic diversity among 32 non redundant accessions. Mean ± standard deviation

Locus	Na[1]	He[2]	Ho[2]	D[3]	PI[4]
VMC1b11	10	0.740	0.750	0.850	0.104
VMC4f3-1	10	0.850	0.875	0.936	0.039
VVIb01	5	0.500	0.656	0.596	0.324
VVIh54	6	0.648	0.688	0.811	0.179
VVIn16	4	0.647	0.719	0.768	0.198
VVIn73	3	0.382	0.438	0.119	0.444
VVIp31	10	0.796	0.938	0.896	0.068
VVIp60	6	0.653	0.625	0.797	0.184
VVIq52	3	0.626	0.594	0.789	0.213
VVIv37	7	0.808	0.906	0.912	0.063
VVIv67	9	0.813	0.938	0.920	0.057
VVMD21	5	0.703	0.656	0.834	0.147
VVMD24	6	0.752	0.906	0.820	0.102
VVMD25	6	0.648	0.594	0.832	0.177
VVMD27	6	0.755	0.844	0.883	0.091
VVMD28	9	0.699	0.813	0.781	0.123
VVMD32	7	0.768	0.781	0.850	0.089
VVMD5	8	0.844	0.875	0.926	0.044
VVMD7	7	0.718	0.813	0.846	0.119
VVS2	9	0.775	0.813	0.895	0.086
Mean	6.8 ± 2.2	0.706 ± 0.111	0.761 ± 0.133	0.803	0.142
Cumulative	136			0.999999999994	2.322 x 10^{-19}

[1] Number of allele per locus (Na)

[2] Expected (He) and observed heterozygosity (Ho)

[3] Discrimination Power (D)

[4] Probability of identity (PI)

The dendrogram revealed 10 bootstrap values above 70%. Four main groups labeled A, B, C and D were found (Figure 2, Table 5). Clusters A and B mainly grouped all the samples related with Callet Cas Concos variety (Lineage 2; Figure 1). Most of these varieties carried chlorotype A, but five (Giró Ros, Sabaté, Excursach, Valenci Tinto and Fogoneu) showed chlorotype D. In these groups only one bootstrap value was consistent (>70%) with the parent relationship found with FaMoz (Valent blanc - Giró Ros). Clusters C and D grouped the other samples, most of them related with Hebén variety (Lineage 1; Figure 1). The varieties carrying chlorotype A were, again, the most frequent ones, although four varieties (Unknown 2, Fernandella, Mancés de Capdell and

Aspiran) showed chlorotype D, and other two varieties carried chlorotype B (Chasselas cioutat and Isabelle). In these groups, four bootstrap values were higher than 70% at terminal nodes and showed parent relationship consistent with FaMoz results (Macabeo – Brustiano faux, Viñaté – Albillo Mayor, Gorgollassa – Mandón, and Santa Magdalena – Legiruela).

Table 5. Assignments of the unique genotypes of the cultivars and proposed parentages (*) following the Dendrogram and Structure cluster assignment (>70% ancestry) results

Genotype	Prime Name used at VGB "Finca El Encín"	Cluster assignment	
		Dendrogram group	Structure cluster assignment
1	Argamusa	A	
2	Batista	D	1
3	Beba	B	3
4	Valenci Tinto	B	
5	Callet	B	3
6	Eperó de Gall	D	2
7	Excursach	B	
8	Fernandella	D	1
9	Fogoneu	B	3
10	Mandón	D	2
11	Mancés de Capdell	D	
12	Gorgollassa	D	2
13	Mansés de Tibbus	D	
14	Manto Negro	A	3
15	Pampolat girat	D	
16	Quigat	D	
17	Sabaté	A	
18	Viñaté	C	1
19	Isabelle	D	1
20	Chasselas cioutat	D	1
21	Santa Magdalena	D	1
22	Tinto Velasco	D	1
23	Callet Cas Concos	B	3
24	Unknown 1	B	3
25	Valent Blanc	A	
26	Gafarró	B	3
27	Giró Ros	A	3
28	Unknown 2	C	
29	Pensal Blanca	A	
30	Unknown 3	A	3
31	Bobal	C	1
32	Macabeo	C	1
*	Albaranzeuli bianco	A	
*	Albillo Mayor	C	1
*	Aspiran	D	
*	Brustiano faux	C	1
*	Planta Fina de Pedralba	C	
*	Graciano	D	
*	Hebén	C	
*	Legiruela	D	1
*	Monastrell	D	2

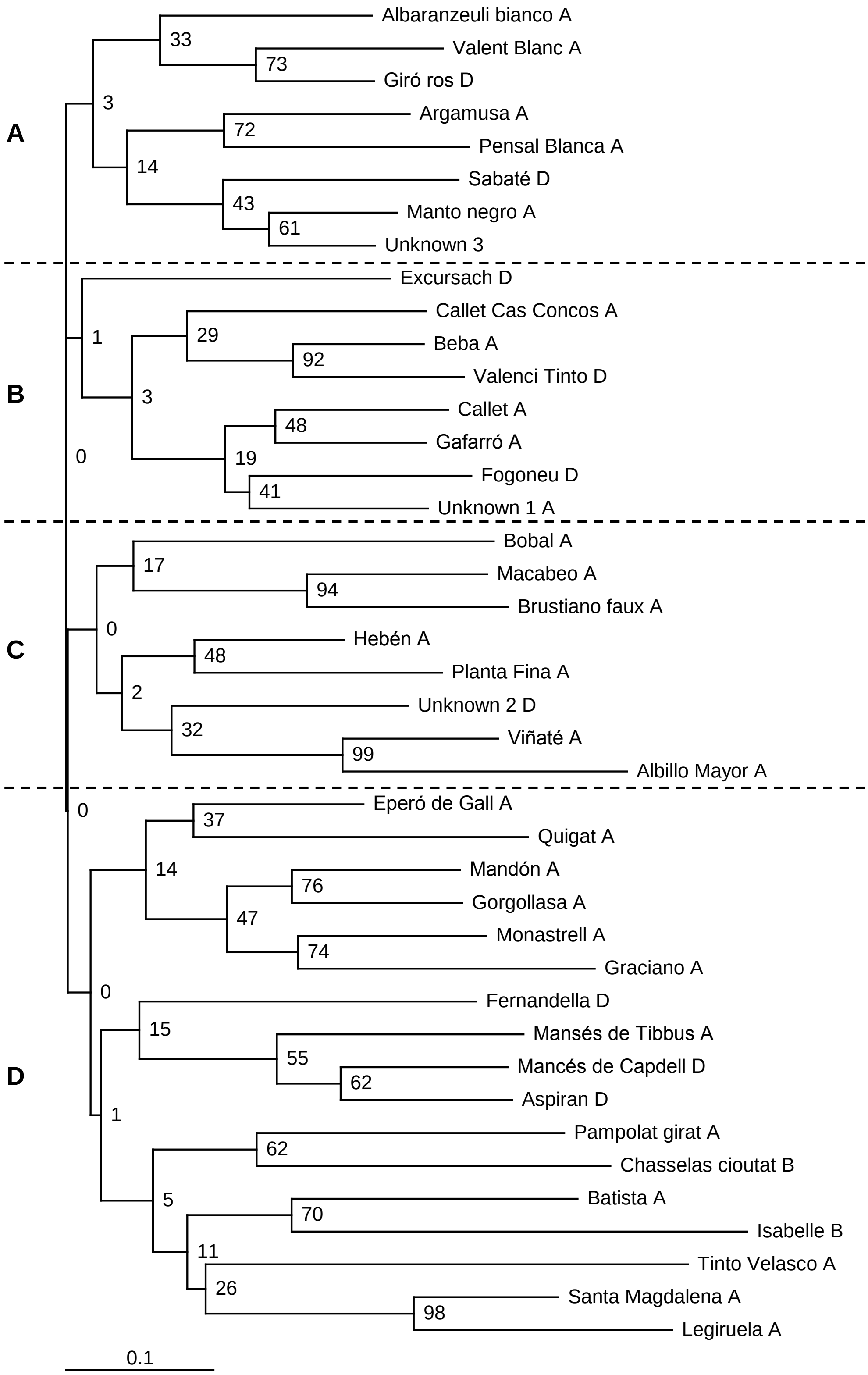

Figure 2. Neighbour-joining dendrogram of genetic relationship among the 32 varieties investigated and their putative parent relationship. Results calculated with Da genetic distance (Nei et al. 1983). Capital letter at the end of the varietal name is the Chlorotype according to Arroyo-García et al. (2006; Table 2)

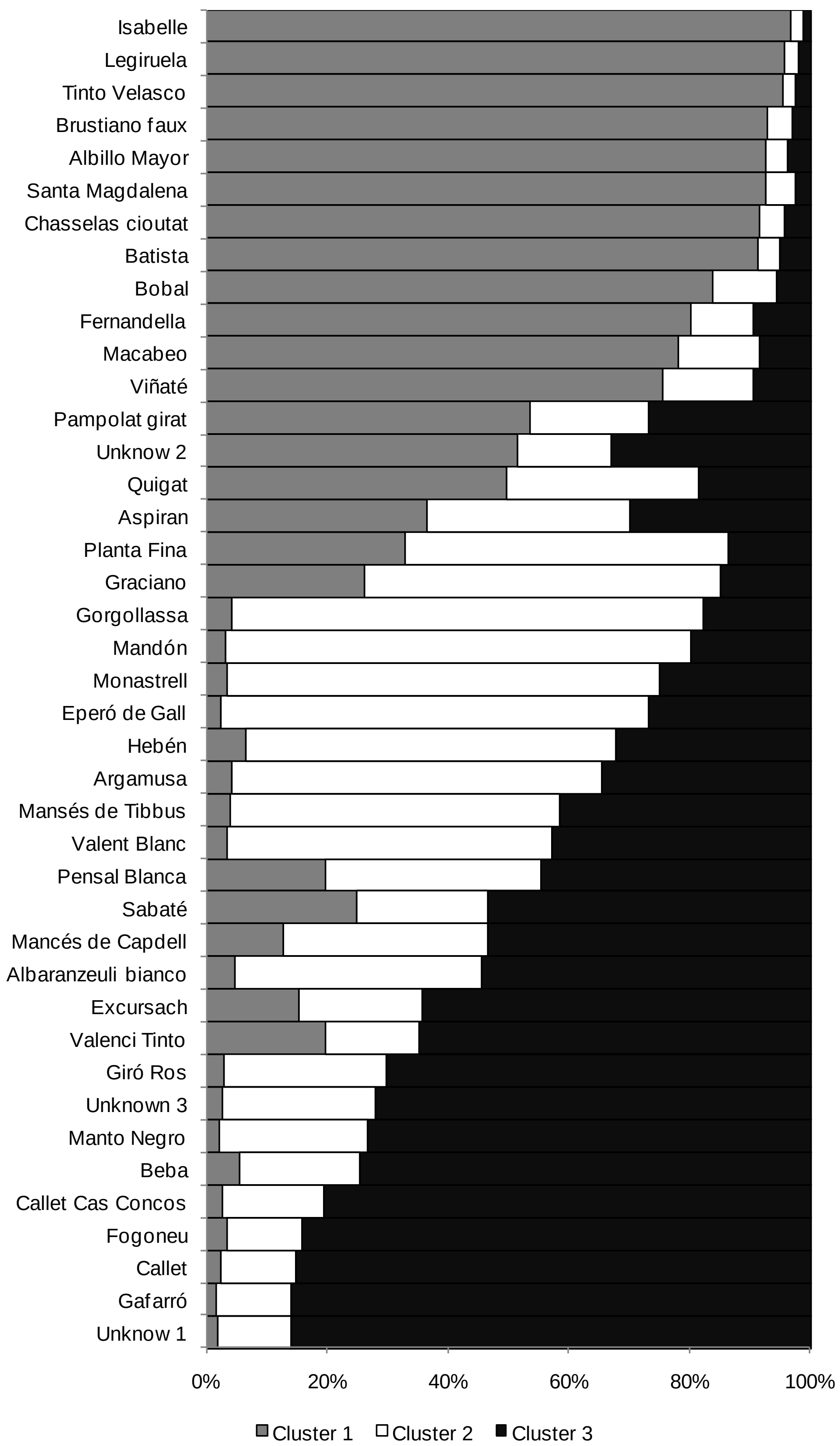

Figure 3. Proportions of ancestry of individual genotypes at 19 SSRs in each of K=3 genetic clusters estimated with Structure software

Structure clustering was applied to confirm these previous results. The optimal number of genetic clusters was K=3 (Ln P(D)=-2287.10; ΔK=24.21). Cluster 1 (12 genotypes) and Cluster 2 (four genotypes) were included in the Clusters C and D found in the neighbour-joining tree. All varieties included in the Cluster 2 but Monastrell shared the variety Hebén as female parent. Cluster 3 (nine genotypes) were distributed among the groups A and B of the dendrogram, grouping the cultivars related with Callet Cas Concos variety, since they shared the half of their alleles in all the loci studied, confirming first-degree genetic relationships. Sixteen varieties were unassigned (Figure 3; Table 5). The genetic diversity parameters studied (Na, He, and As) for clusters characterization were higher for Cluster 1 (Table 6) being the most heterogeneous group. All differentiation tests among pairs of clusters were significant ($p<0.001$) being the greatest differences between Clusters 2 and 3 ($F_{ST}=0.140$; Table 7). This was also confirmed by multivariate analysis (FCA). All varieties were assigned in the original group established with Structure software (Figure 4).

Table 6. Genetic diversity among the 3 Clusters for 20 SSR identified with Structure software. Mean ± standard deviation

	n[1]	Na[2]	He[3]	Ho[3]	As[4]
Cluster 1	12	6.0 ± 1.8	0.728 ± 0.098	0.750 ± 0.151	4.09 ± 0.83
Cluster 2	4	2.8 ± 1.0	0.506 ± 0.233	0.725 ± 0.352	2.80 ± 1.03
Cluster 3	9	3.4 ± 1.0	0.611 ± 0.098	0.802 ± 0.131	3.00 ± 0.70

[1] Number of varieties included in each cluster (n)

[2] Number of allele (Na)

[3] Expected (He) and observed (Ho) heterozygosity

[4] Allele richness (As)

Table 7. Pairwise F_{ST} values for the Clusters identified with Structure software (above the diagonal) and *p* values (in the lower triangle) obtained with ARLEQUIN and tested with FSTAT

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	-	0.095	0.079
Cluster 2	<0.001	-	0.140
Cluster 3	<0.001	<0.001	-

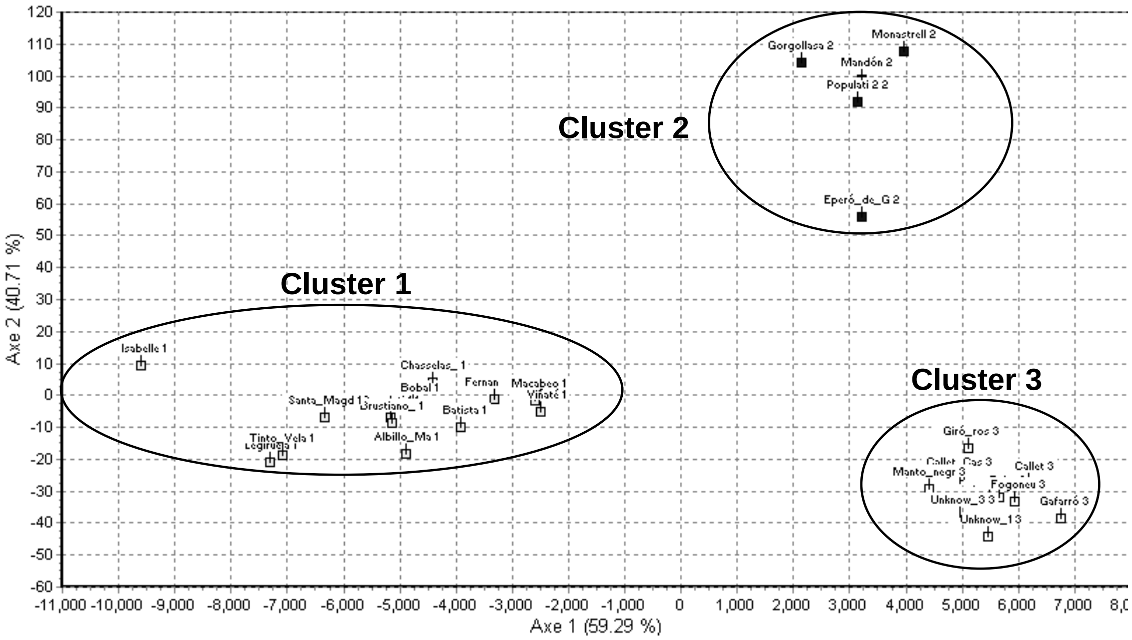

Figure 4. Factorial component analysis based on the genetic clusters estimated with Structure

Discussion

Identifications, synonyms, homonyms

The identical genotypes found in this work could be strongly considered as synonyms since the cumulative probability of identity is around 10^{-19} (Sefc et al. 2000). We confirmed the synonym between Batista and Canari found by Truel (1985) and found four other new synonyms for Excursach, Mansés de Capdell, Giró Ros, and Pampolat girat matching with varieties located around the Mediterranean basin. The varieties Unknown 2 and Unknown 3 have different genotypes, although they were named Manto Negro, confirming that under the Manto Negro designation were identified several varieties (Oliver Moragues 2000). These findings corroborate that the mistakes are very common as a consequence of the high number of cultivated grape varieties and variability of ampelographic characters (Vouillamoz et al. 2004; Martínez et al. 2009).

The Beba variety is usually used as white table grape. It shows a high number of synonyms being Calop the most antique (Martí 1978). Other synonyms have been found as Ain el Kelb and Tebourbi (Laiadi et al. 2009) or Panse de Provence (Truel 1985), confirming the high historic value of this variety through time. There is a rose mutation of Beba, called Calop rojo and Calop roig. However, between the white and rose accessions of Beba variety no differences were found either with the nuSSRs nor with ampelographic descriptions except the berry colour (Chapter 3). Thus Beba rose is a somatic mutation of the white Beba and they could be considered as different cultivars of the same genotype.

The Batista variety is present in Spain only in the Balearic Islands but it was found in the South Western of France and in Italian Alpes under Canari and Luverdon names (Schneider et al. 2001).

Pampolat girat is found in the Balearic Islands, Valencia and Catalonia, at least, since 1845 (Odart 1845) and it was found in Corsica too (France). Its synonym Cruixent is present in the Luxembourg Catalogue (Viala and Vermorel 1910). Whereas Excursach is a female variety found in the Balearic Islands almost since 1842 (Ferrer 1999). Its synonym Murescu (*vs* Muresca) has been mentioned in Corsica in 1822 (CIVAM 1992).

The first reference found for the Giró variety corresponds with a map of the Balearic Islands (Despuig 1784). Under the names of Giró two different varieties are known, with different genotype, the black varieties Mansés de Capdell (syn. Giró and Mancens) and Giró Ros (syn. Giró sardo), although a white cultivar was found for Giró Ros (syn. Giró sardo) in the Balearic Islands. Our results showed that Giró Ros have the same genotype for white and black cultivars, it means that a somatic mutation exist, therefore they could be considered as different cultivars.

The Mansés de Capdell (syn. Giró and Mances) variety was present in the Balearic Islands and Catalonia as Mancés, Mansés (Carretero 1875) or Mancesa (Abela y Sáinz de Andino 1885), and in the French Oriental Pyrenees as Mancens (Truel 1985). The Giró Ros (syn Giró sardo) variety was present in the Balearic Islands (Estelrich 1903) and in Sardinia (Italy; Ottavi 1868).

Geographical distribution

The synonyms and homonyms are the result of migration events and cultural exchanges (Labra et al. 2002), lack of ampelographic knowledge and adaptation or substitution of the native names in different languages for the unfamiliar varieties (Aradhya et al. 2003). Therefore, the geographical origin of the grapevine is complex to establish (This et al. 2006), although the haplotypic distribution on a large sample is a useful tool to suggest grapevine origin (Imazio et al. 2006). Haplotypes percentage found in this work was similar to the one described by Arroyo-García et al. (2006); they found that chlorotype A was the most frequent one in the cultivated grapevine in the Central and West part of the Mediterranean basin; whereas the clorotype B was the least frequent one. The Excursach, Mansés de Capdell and Giró Ros varieties might have a Greek ancestral attending to their chlorotypes. They showed chlorotype D, the most frequent one in Greece (Arroyo-García et al. 2002) and in the Italian and Balkan Peninsulas (Arroyo-García et al. 2006). This could explain the high assignment rate from Greek cultivars to Spanish ones found by Sefc et al. (2000).

The migration of some of the studied varieties seems to have occurred in three different periods. The first one could be considered around VII century with the expansion of Islam. Although the origin of Beba variety is unknown, the oriental or North African origin has been suggested (Laiadi et al. 2009). This variety is present in Spain especially around the Mediterranean coast (Valcárcel 1791) as well as in Algeria (Pulliat 1898). The etymology of the Beba name in Spanish could come from Arabic or Hebrew (Rojas Clemente 1879). However a

Spanish origin of this variety in Algerian viticulture has been suggested by Pulliat (1898) under the name of Valenci, which remember the name of Valencia (Spain). This theory is possible as a consequence of the important emigration from the Balearic Islands to Algeria after the phylloxera crisis, during the XIX century (Oliver Fuster 1980). The Excursach variety could be connected with Moors (CIVAM 1992) supported by the female ancestral trait of Excursach. This variety is also found in Corsica, so it might be spread by Moors both in Corsica and the Balearic Islands, giving birth to Fogoneu in the Spanish islands, which is not present in France or Italy. An alternative explanation suggests that the Spanish could have carried or brought this variety from or to Corsica, during XIII-XV centuries, when Corsica, Sardinia and the Roussillon were under the Kingdom of Aragón (current Spain). This last theory could also be proposed for Pampolat girat, although the Corsican name Cruixent, written with “x”, letter rarely included in Corsican and French alphabets but frequent in Catalonian one, make us to hypothesize that this variety could have been carried from Spain to Corsica. The Mansés de Capdell (syn. Giró and Mances) variety seems to be related too with Fogoneu; hence we think that this variety was first in Spain and then carried to Oriental Pyrenees. The Giró Ros (syn Giró sardo) origin is uncertain; it could have been first in Spain and then brought to Sardinia when they were under Kingdom of Aragón (current Spain) since this variety is related with Callet Cas Concos variety. This theory is supported by the ancient trait of the color mutation found for this cultivar, as well as the Spanish origin of this cultivar in Sardinia has been previously mentioned (OIV 1961). The influence of the Spanish in Sardinian viticulture has been described by de Mattia et al. (2007), given that the Sardinian cultivars are related with Italian and Spanish varieties (Grassi et al. 2003). However, attending to its name (Sardo, from Sardinia) it could have been first present in Sardinia and then brought to the Balearic Islands.

Batista origin is uncertain although it seems to have a French origin (Schneider et al. 2001). Nowadays this variety is present in Spain only in the Balearic Islands. It might be carried from France to Catalonia and then to the Balearic Islands when the Roussillon was under the County of Catalonia (current Spain) around the XII century. Figure 5 summarize those entire hypotheses.

Parentage analysis

The bibliography confirmed the coexistence between the proposed parental. The cpSSRs are maternally inherited in grapevine (Arroyo-García et al. 2002) revealing the direction of the cross. Only in Giró Ros=Valent Blanc x Albaranzauli bianco an incompatibility of chlorotype was found. In one case, Santa Magdalena=Planta fina de Pedralba (syn. Farana, Mayorquin) x Legiruela (syn. Prié blanc, Agostenga), all the cultivars showed the same chlorotype, therefore the female and male parents could not be specified; the bootstrap value of the dendrogram suggested a strong genetic relationship between the cultivars. Other direct relationships for Legiruela (Albillo Real and Luglienga bianca) have been described (Schneider et al. 2010). On the basis of LOD score and the historical references the parentage Fogoneu=Excursach x Mansés de Capdell is the most likely; although Aspiran variety is related with Mansés de Capdell as they shared half of their alleles.

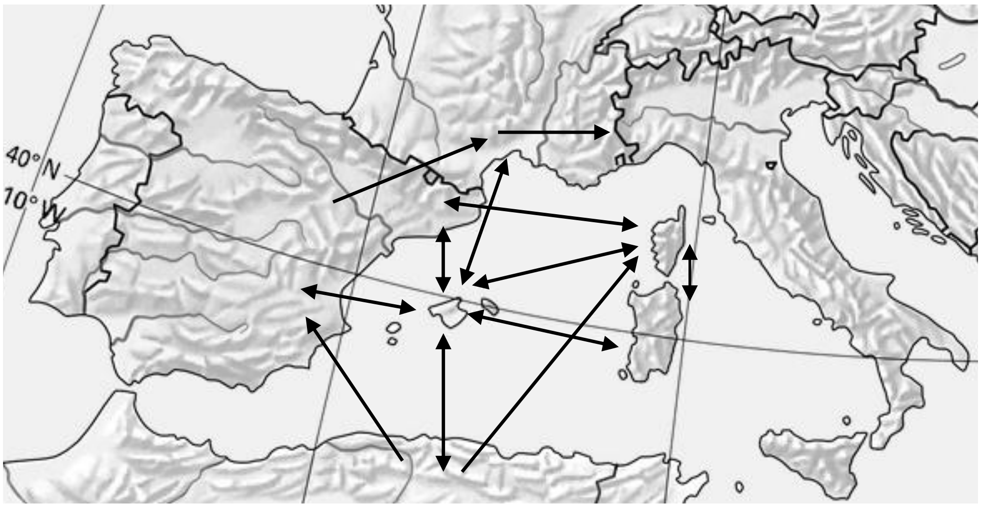

Figure 5. Theories about the movement of the studied cultivars through history (explained in the text)

Several crosses were inconsistent with one nuSSR marker since a discrepancy of three loci was fixed to assign the parentage allowing possible mistakes, which may be due to the presence of null alleles or mutations (Boursiquot et al. 2009, and references therein). We obtained several putative parentages, six of them with full compatibility for all nuclear microsatellites studied and for the chlorotype of both parents, sustaining the consistency of the suggested pedigrees.

Genetic structure

The VMC4f3-1and VVIn73 nuSSRs have been already described to be respectively the most and least informative ones by other authors (El Oualkadi et al. 2009; Riahi et al. 2010) since a higher discrimination power (D) implicates a lower probability of confusion of cultivars (Sefc et al. 2000). The expected heterozygosity is a useful measurement to compare with other works since the number of allele per locus is sensitive to the number of cultivars analyzed (Sefc et al. 2000; Aradhya et al. 2003). The genetic diversity found in this work (70.6%) was similar to the one obtained in studies with analogous sample size (de Mattia et al. 2007; Santana et al. 2008).

The different clustering methods used are consistent with the existence of at least two lineages in the analyzed samples. The greatest differences between Clusters 2 and 3 are justified since the Cluster 2 could perform the first gene pool, since the most of the varieties are related with Heben variety, and Cluster 3 could perform the second one, being Callet Cas Concos the key variety. This cultivar is related with two of the most important wine varieties in the Balearic Islands (Callet and Manto Negro).

Conclusions

Material exchanges of grapevine are related to historical migrations of civilizations consequently giving several synonyms around the Mediterranean basin. The geographic distribution proposed for the varieties from the Balearic Islands are consistent with their historical references.

Prospection are useful and interesting for the discovery of new genotypes and they are also needed in order to ensure the long-term survival of the found cultivars. The finding of Callet Cas Concos and Hebén varieties was the key for clarifying several crosses. Unknown varieties, under risk of extinction, are putative options for breeder, for the wine industry and could clarify new genetic relationships. Finally, the combination of ampelography, microsatellite analysis and synthesis of historical references of the cultivars has shown to be excellent tools for a good identified grapevine material and to establish a correct regional viticulture.

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Chapter 5

Danger

(This photo is courtesy of Gregorio Muñoz Organero)

Evaluation of susceptibility to powdery mildew (*Erysiphe necator*) in *Vitis vinifera* varieties

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Abstract

Susceptibility to grape powdery mildew (*Erysiphe necator* Schwein.) was studied in 159 *Vitis vinifera* foreign and native grape varieties grown in Spain. The relationship between morphological features of vines and their susceptibility to the disease was also studied. The infection was evaluated under natural conditions on leaves and bunches. A total of 35 cultivars were very susceptible to the disease (very low to low resistance on bunches), while another 83 showed low susceptibility (high to very high resistance on bunches). Results provide useful information for grape growers and breeders for the selection of varieties less susceptible to powdery mildew.

Key words: *Erysiphe necator*, morphology, *Oidium*, susceptibility, *Vitis vinifera*

Introduction

Fungal diseases are a major problem in the cultivation of grapevine, and one of the most threatening pathogens is the fungus *Erysiphe necator* Schwein., the casual agent of powdery mildew. This biotrophic ascomycete invades host epidermal cells and colonizes leaves, rachis, and grapes, causing a decrease of vine growth, yield, and quality of grapevine production (Pool et al. 1984, Calonnec et al. 2004). The incidence of powdery mildew has increased in recent years in Europe. Climatic conditions and reduced efficacy of fungicides have been suggested as possible reasons (Staudt 1997). Fungicide treatments increase economic costs and negatively affect the environment. Furthermore, fungal strains are developing resistance to some commonly used fungicides (Savocchia et al. 2004). Thus, the possibility of selecting less-susceptible, high-quality cultivars is an alternative management strategy of great importance. Although the most commonly cultivated species, *Vitis vinifera*, has proved to lack resistance to powdery mildew, the degree of susceptibility varies with the cultivar and the environmental conditions (Li 1993; Péros et al. 2006).

The aims of the present study were to analyse the susceptibility of 159 cultivars of *Vitis vinifera* to powdery mildew and to determine whether morphological features may influence this response on the vine.

Material and Methods

Material

This study was conducted for four years (2006-2009). Vines were located in the *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain). Some clones of each variety were studied to determine the degree of susceptibility to powdery mildew, resulting in 473 samples from 159 cultivars (2–7 clones per cultivar, 4 plants per clone; Table1).

Table 1. Modal data of maximum degrees of resistance to powdery mildew on bunches (B) and leaves (L) of *Vitis vinifera* cultivars, according to OIV descriptors 455 and 456: l (low)=1, 3; m (medium)=5; h (high)=7, 9. The cultivars are listed in order based on the resistance to the fungus on bunch, following by the resistance on leaf and alphabetical order. The varieties collected in the Balearic Islands are in bold

Variety	B	L
Beba	l	l
Benedicto de Aragón	l	l
Brancellao	l	l
Cabernet-Sauvignon	l	l
Castellana Blanca	l	l
Espadeiro	l	l
Forastera	l	l
Garnacha Blanca	l	l
Garnacha Tintorera	l	l
Gualarido	l	l
Cayetana Blanca	l	l
Malvasía Aromática	l	l
Marfal	l	l
Mazuela	l	l
Mencía	l	l
Moscatel de Grano Menudo	l	l
Parraleta	l	l
Planta Fina	l	l
Rocía	l	l
Salceño Blanco	l	l
Sumoll	l	l
Tempranillo	l	l
Verdil	l	l
Vidadillo	l	l
Benedicto	l	m
Cariñena Blanca	l	m
Garnacha Tinta	l	m
Godello	l	m
Morisca	l	m
Palomino	l	m
Palomino Fino	l	m
Picapoll	l	m
Rey	l	m
Sabro	l	m
Torrontés	l	m
Albillo Mayor	m	l
Beba roja	m	l
Cagarrizo	m	l
Cañorroyo	m	l
Chasselas Doré	m	l
Moscatel de Alejandría	m	l
Juan García	m	l
Pedro Luis	m	l
Pedro Ximénez	m	l
Planta Nova	m	l
Salvador	m	l
Zalema	m	l
Airén	m	m
Alarije	m	m
Albillo de Albacete	m	m
Albillo Real de Granada	m	m
Borba	m	m
Chenín Blanc	m	m
Doña Blanca	m	m
Garnacha Roja	m	m
Garrido Fino	m	m
Garrido Macho	m	m
Graciano	m	m
Listán del Condado	m	m
Macabeo	m	m
Merenzao	m	m
Morate	m	m
Puesto Mayor	m	m
Rojal Tinta	m	m
Rufete	m	m
Savagnin Blanc	m	m
Tetona	m	m
Treixadura	m	m
Verdejo	m	m
Verdejo Tinto	m	m
Bastardo Negro	m	h
Bobal	m	h
Excursach	m	h
Fernandella	m	h
Gabriela	m	h
Tarragoní	m	h
Alcañón	h	l
Batista	h	l
Cabernet Franc	h	l
Derechero	h	l
Garnacha Peluda	h	l
Grumet	h	l
Hebén	h	l
Malvar	h	l
Maturana Blanca	h	l
Moscatel de Grano Gordo Rosa	h	l
Prieto Picudo	h	l
Puerto Alto	h	l
Verdejo de Salamanca	h	l
Xarel.lo	h	l
Albariño	h	m
Allarén	h	m
Argamusa	h	m
Valenci Tinto	h	m
Moravia Dulce	h	m
Caíño Tinto	h	m
Caíño Bravo	h	m
Callet	h	m
Chasselas Rosé	h	m
Cherta	h	m
Ferrón	h	m
Folle Blanc	h	m
Fumat	h	m
Giró	h	m
Jaén Rosado	h	m
Jaén tinto	h	m
Listán Negro	h	m
Mantúo	h	m
Mantúo de Pilas	h	m
Merseguera	h	m
Monastrell	h	m
Moravia Agria	h	m
Morenillo	h	m
Moristel	h	m
Moscatel de Angüés	h	m
Negramoll	h	m
Pardillo	h	m
Rayada Melonera	h	m
Sinsó	h	m
Sousón	h	m
Tinto Velasco	h	m
Tortosí	h	m
Trepat	h	m
Vijariego Blanco	h	m
Xarel.lo Rosado	h	m
Albillo Real	h	h
Blanquiliña	h	h
Carrasquín	h	h
Cuatendrá	h	h
Doradilla	h	h
Eperó de Gall	h	h
Fogoneu	h	h
Forcallat Tinto	h	h
Gorgollassa	h	h
Legiruela	h	h
Listán Prieto	h	h
Loureira	h	h
Malvasía Volcánica	h	h
Mandón	h	h
Mansés de Tibbus	h	h
Manto Negro	h	h
Mondragón	h	h
Morrastel-Bouschet	h	h
Ondarrabi Beltza	h	h
Pampolat de Sagunto	h	h
Pampolat Girat	h	h
Parduca	h	h
Parellada	h	h
Pedrol	h	h
Perruno	h	h
Petit Bouschet	h	h
Quigat	h	h
Sabaté	h	h
Santa Magdalena	h	h
Señá	h	h
Trobat	h	h
Verués de Huarte	h	h
Viñaté	h	h

All plants were grafted onto 41B and were almost 30 years old. The plantation compass was 2.5 m × 2.5 m. Repetitions of the cultivars were randomly arranged in the same plot. Therefore, all the cultivars were subjected to the same edapho-climatic conditions and traditional management practices. They were cultivated in dry land, with a training vessel and had no phytosanitary treatment during the period of study. Mazuela (synonyms Cariñena, Carignan Noir) was used as the susceptibility control and it was regularly distributed in twelve different locations along the plot to control the uniformity of the infection.

Methods

Climatic data were recorded for the years of the study. Evaluation of natural infection was performed from June to September (about 3 weeks after onset of flowering for leaves and before vintage for bunches). Infection levels were visually estimated following the descriptors OIV-455 and OIV-456 of the International Organization of Vine and Wine (OIV 2007), which refer to the degree of resistance on leaves and bunches, respectively, using a 1 to 9 scale (1=very low resistance, 9=high resistance). The cultivars were classified into three classes, depending on the level of these descriptors, as follows: levels 1–3, a low or very low degree of resistance (high susceptibility); level 5, a medium degree of resistance (medium susceptibility); and levels 7–9, a high to very high degree of resistance (low susceptibility).

The following morphological characters of leaves and bunches were selected from the OIV descriptors list (OIV 2007) to identify factors associated with resistance to powdery mildew: (I) Young leaf: OIV-051 and OIV-053, (II) Mature leaf: OIV-065, OIV-072, OIV-075, OIV-084 and OIV-087, (III) Bunch: OIV-202, OIV-203, OIV-204 and OIV-208, and (IV) Berry: OIV-220, OIV-221 and OIV-223. Spearman Rho coefficients for ranked data were calculated to detect all possible correlations between morphological and disease variables. All statistical analyses were performed with the statistical program SPSS v.15.

Results and discussion

For all of the years, the climatic conditions seem to be favourable for the *Erysiphe necator* development (Figure 1). Intraspecific variation in the susceptibility to powdery mildew has been found among the studied *Vitis vinifera* cultivars as other authors have reported (Doster and Schanathorst 1985; Li 1993; Eibach 1994). The most frequent level of susceptibility observed was the medium level (35-50% of the varieties, depending on years), which corresponds to leaves with attacked patches, usually limited to a diameter of 2 to 5 cm, many attacked berries (up to 30 %), and most clusters moderately attacked. Between 13% and 52% of the varieties were very susceptible to powdery mildew on bunches, showing many berries of all clusters attacked and many cracked berries. On the other hand, between 13% and 36% of the cultivars showed bunches with low susceptibility. In these cases, only a few berries of all clusters were attacked.

The maximum degree of resistance for each cultivar and year was calculated, and the modal data were obtained for the whole period (Table1). A total of 35 cultivars (22%) were very susceptible to the disease (very low–low resistance on bunches), while another 83 (52.2%) showed low susceptibility (high to very high resistance on bunches).

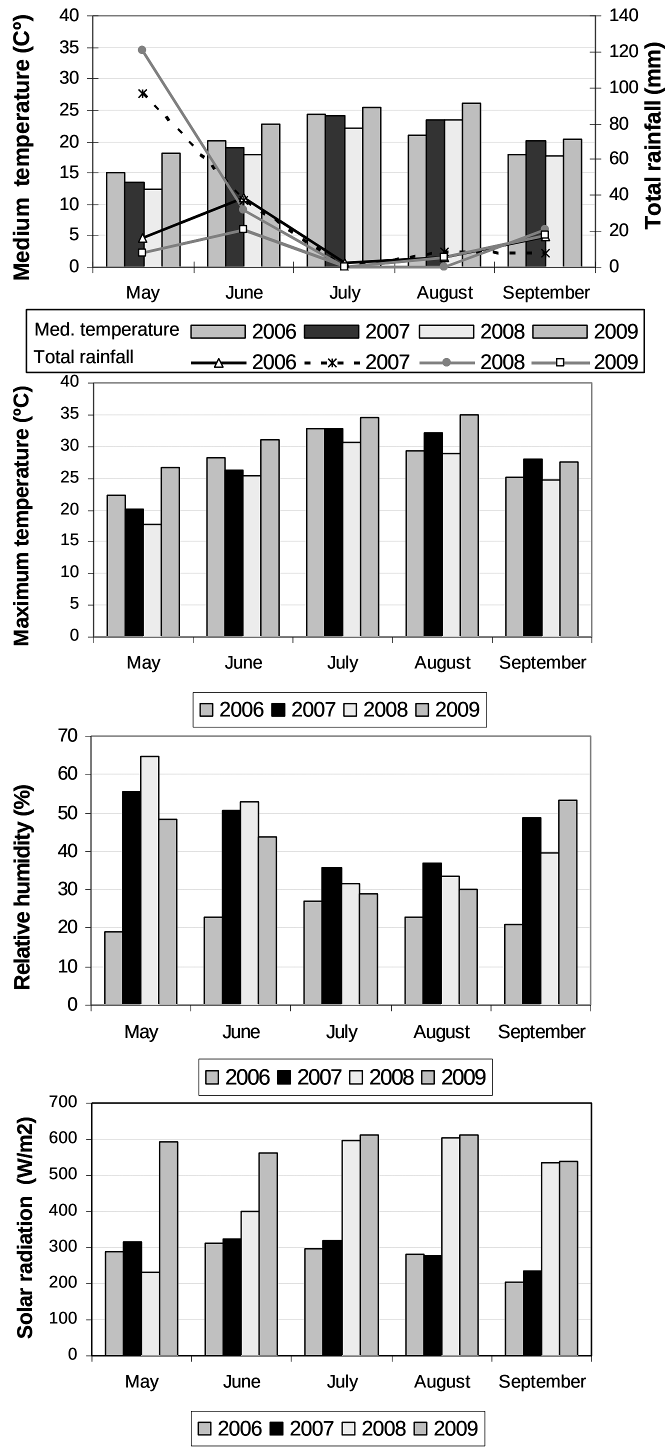

Figure 1. Climatic data during the period of study (2006-2009)

The variety used as the susceptibility control, Mazuela, has proved to be very susceptible to powdery mildew, as other authors have found (Doster and Schnathorst 1985; Li 1993; Péros et al. 2006). Li (1993) and Péros et al. (2006) also noted a high susceptibility of Cabernet-Sauvignon, close to the response of Mazuela and the same results were obtained in this study. On the other hand, Grenache (synonym Garnacha Tinta) has been reported to be less susceptible than Cabernet-Sauvignon (Boubals 1961; Li 1993); however, our results corroborated this finding only in the case of susceptibility on leaves because both cultivars showed the same results for bunches. Péros et al. (2006) found likewise that Grenache was not always distinguished from very susceptible cultivars in laboratory tests. Results for Folle Blanc (syn. Folle Blanche), Monastrell (syn. Mourvèdre), Macabeo (syn. Maccabeu), and Garnacha Tintorera (syn. Alicante Bouschet) have been also reported by Li (1993). We agree with his findings that Folle Blanc and Monastrell showed a medium level of susceptibility with respect to Mazuela, which was lower in the case of Garnacha Tintorera. In contrast to values obtained by Li (1993), we observed a minor level of susceptibility for Macabeo relative to the control, which could be due to the existence of diverse homonyms for Macabeo.

Results showed that the degree of resistance to powdery mildew on leaves correlates positively with resistance on bunches. This correlation was high in 2008 (r=0.75), when the degree of infection was also higher, probably because of the most favourable climatic conditions having occurred in that year. The coefficient of determination indicated that 56% of the variation in the berry resistance could be explained by variation in the leaf resistance in that year. However, this correlation was smaller in 2007 (r=0.52) and 2009 (r=0.38), verifying that the relationship between variables is not sufficiently consistent in time and may depends on the fungal infection pressure.

Previous studies have demonstrated a relationship between morphological features and the susceptibility of *Vitis vinifera* cultivars to powdery mildew. In our study, only two out of the fourteen ampelographic characters studied were significantly correlated with resistance to powdery mildew. We detected a significant negative correlation between the degree of resistance on leaves and the goffering of blades (r=-0.16 to -0.22) and between bunch density and the degree of resistance on berries (r=-0.17). These two effects could be related with a less ventilation of these types of leaves and bunches altering microclimatic conditions to favour fungal development. However, the low correlations obtained do not show a strong relationship. Therefore, the selection of these morphological characters will not assure resistance in the selected cultivars.

Conclusions

Knowledge about the degree of susceptibility of each variety makes the selection of less susceptible cultivars possible for grape growers. This is an important advantage, especially in areas where climatic conditions are often favourable for the disease, such as the Mediterranean region. Thus, fungicide treatments may be substantially reduced in an important number of cultivars, enabling a reduction in economic and environmental costs.

Acknowledgements

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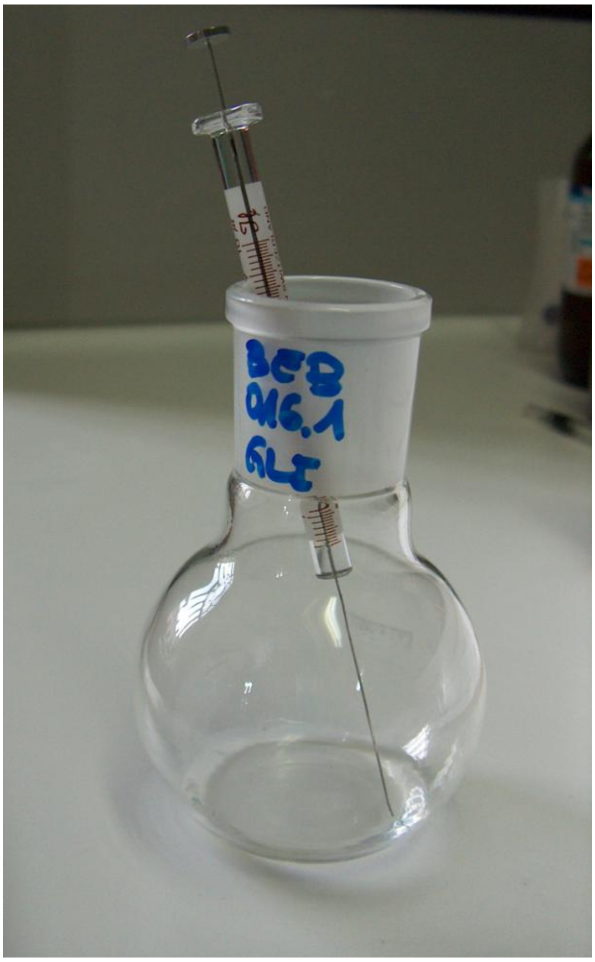
Chapter 6



A tear full of aroma

Aromatic characterization and oenological potential of 21 minor varieties (*Vitis vinifera* L.)

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Abstract

The homogenization of international wine market led to a gradual impoverishment of the genetic pool. In fact, in several Spanish Quality Demarcations, the most frequently international varieties are replacing the local ones. As a result, minor varieties, perfectly adapted to the local environmental conditions, are nowadays at risk of extinction. The study of minor varieties could provide useful inputs to satisfy the demand for new and interesting wine products. This work aims at filling a gap in the existing literature, focusing on the aromatic potential of minor varieties. Here, the study of glycosidic volatile compounds and the evaluation of the influence of several variables on aroma composition were considered. Fifty-one glycosidic compounds were identified and quantified. In order to identify the most powerful aroma compounds of the studies grapevines, the Odour Activity Values (OAVs) was used. Floral, spicy and phenolic were the most important odorant series of OAV evaluation. The results revealed differences for glycosidic compounds according to cultivars, berry colour, clone and sample origin. Moreover the synthesis of some compounds involved in these differentiations seems to have a genetic component. The characterization of aromatic potential of several minor varieties, achieved for the first time, revealed that some of those (Argamusa, Gorgollassa and Pampolat girat) could represent an excellent option for winemaking and commercial offer diversification strategies, besides being important for these cultivars conservation.

Key words: Aroma, glycosides, gas-chromatography, flavour

Introduction

The phenomenon of replacement of local grape varieties with widely-spread international cultivars, such as Cabernet-Sauvignon or Chardonnay, is coming to a standstill. In addition, the wine consumers taste and preferences have changed during the last few years, since there are other values and motivations out of aroma and taste for drinking wines, e.g. as marketing attributes and new wine style (Lesschaeve 2007). Several Quality Demarcations are starting to promote varieties linked to specific locations, which produce original and high quality wines. Minor varieties, perfectly adapted to the local environmental conditions, may represent a good option. Even though they may not be allowed in any Quality Demarcation; their reintroduction in the wine industry would not only entail an increase in the wine offer, but also provide an important tool for their conservation. Being the wine aroma one of the most important attributes for influencing consumer´s preferences, a more detailed study about the aromatic potential of these traditional varieties appears to be an important first step.

The qualitative and quantitative volatile compounds in grapes are important as a source of flavour in wines (Fang and Qian 2006). The grape and wine aroma, and specifically its intensity, has long been one of the principal attributes taken into account for grapevine selection (Duchêne et al. 2009b). The wine aroma compounds depend on the cultivar (Ferreira et al. 2000; Esti and Tamborra 2006), on environmental conditions (Ribéreau-Gayon et al. 2006) as well as on oenological processes such as maceration (Peinado et al. 2004), yeast and fermentation (Loscos et al. 2007; Ugliano and Moio 2008).

The aromatic compounds have been studied in several grape varieties (Cabrita et al. 2006), as well as the relationship between the grape variety and the wine obtained (Mulet et al. 1992; Ugliano and Moio 2008). In grapes those compounds can be found in their free and bound form, especially as glycosides (Ugliano and Moio 2008). The majority of wines are made with neutral varieties, characterized by very low, and sometimes not detectable, values of the free volatile forms content (Cabrita et al. 2006). Consequently, the contribution of those compounds to the final wine aroma is often negligible (Ugliano and Moio 2008). By contrast, bound compounds, together with fermentation volatile ones, can contribute to the wine aroma (Ugliano and Moio 2008), since they can be hydrolyzed by yeast or exogenous enzymes during winemaking (Ugliano and Moio 2008; Loscos et al. 2009) and aging (Fang and Qian 2006).

Although all volatile compounds contribute to the odour (Ryan et al. 2008), only a few of them play a role in determining varietals aroma (Rocha et al. 2004). However, the compounds with higher odour activity value (OAV) could define the main odorant notes in musts and wines (Guth 1997; Franco et al. 2004). The selection of these compounds helps understanding their impact on the aroma profile (Lorrain et al. 2006). This research focuses on the glycosidic volatiles, whose quantification could define the potential wine aromatic profile, according to the reviewed literature (Loscos et al. 2009).

Previous studies on the use of aroma compounds in wine characterization have been carried out for Callet, Manto Negro and Fogoneu, varieties included in several Quality Demarcations of the Balearic Islands, Spain (Mulet et al. 1992; Forcen et al. 1993). For the remaining ancient cultivars, the contribution of grape aroma precursor to wine aroma profile has not yet been studied. In this work we analyzed the aromatic potential of 21 varieties, mainly native from the Balearic Islands, Spain. The aims of this study were (i) to characterize the glycosidic compound in grapevine (*Vitis vinifera* L.), (ii) to approach the aroma profiles of obtained wines considering the glycosides compounds with major odour activity value, and (iii) to evaluate how different variables such as the berry colour, the accession (or clone), and the origin of the samples can influence the aroma profile. Furthermore, improving the knowledge of minor varieties could represent an interesting option for wine marketing strategies, aiming at satisfying the evolving consumer´s demand.

Material and Methods

Plant material and field conditions

We analyzed 32 accessions of *Vitis vinifera* L. corresponding to 21 different varieties of *Vitis vinifera* L. since some accessions or clones of the same variety were studied (García-Muñoz et al. 2011). Some of the studied varieties are allowed in several Spanish Quality Demarcation (Table 1). The varieties are preserved at the *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain) and were collected mainly in the Balearic Islands (Spain) but also from the Levante area and Girona (Table 1).

Table 1. List of the accessions analyzed; conserved at the *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain). Acc.: accession; Id.: code used in the analysis; Origin: PM Palma de Mallorca; GI Girona, LE Levante area; N: Number of Spanish Quality Demarcation where the variety is allowed; - Minor varieties non included in a Quality Demarcation; * allowed out of the Balearic Islands

Main name	Acc.	Id.	Berry colour	Origin	N
Argamusa	E34	ARG.E34	white	PM	-
Batista	E23	BAT.E23	red	PM	-
Beba	E39	BEB.E39	white	GI	1*
Beba	O16	BEB.O16	white	LE	1*
Beba	O17	BEB.O17	white	LE	1*
Beba	O44	BEB.O44	white	LE	1*
Beba	E14	BEB.E14	white	PM	1*
Beba	E32	BEB.E32	white	PM	1*
Beba	E43	BEB.E43	white	PM	1*
Beba roja	E19	BER.E19	rose	PM	-
Bobal	E11	BOB.E11	red	PM	6*
Bobal	E21	BOB.E21	red	PM	6*
Callet	E26	CAL.E26	red	PM	2
Eperó de Gall	E37	EPE.E37	red	PM	-
Escursach	E27	EXC.E27	red	PM	-
Fernandella	E22	FER.E22	red	PM	-
Fogoneu	E24	FOG.E24	red	PM	1
Fogoneu	E16	FOG.E16	red	PM	1
Giró	E31	GIR.E31	red	PM	-
Giró	E36	GIR.E36	red	PM	-
Gorgollassa	E25	GOR.E25	red	PM	-
Mansés de Tibbus	E30	MES.E30	red	PM	-
Manto Negro	E29	MTN.E29	red	PM	2
Manto Negro	E20	MTN.E20	red	PM	2
Mandón	E15	GAL.E15	red	PM	-
Pampolat girat	H24	PAM.H24	red	LE	-
Pensal Blanca	SCL	PEN.SCL	white	PM	2
Quigat	E38	QUI.E38	white	PM	-
Quigat	E33	QUI.E33	white	PM	-
Sabaté	E35	SAB.E35	red	PM	-
Valenci Tinto	E18	BEN.E18	red	PM	-
Viñaté	E45	VIN.E45	white	PM	-

The grapes were picked by hand, in excellent sanitary conditions, at commercial maturation during 2007 vintage. Approximately 1.5 kg of grapes were collected from the whole vine, ensuring the selection of a fully representative sample. Three sub-samples of 100 berries were randomly selected from the grape sample from each accession. They were stored in labelled glass containers at -30 ºC until analysis were performed.

Plants were almost 20 years old and were grown under the same field conditions. All of them were unirrigated and grafted onto 41B, trained vessel and eight buds per vine were left in winter pruning. The distance between vines was 2.5 m and the one within rows was 2.5 m.

Sample preparation

Aroma compounds were extracted following a previously described method (Cabrita el al. 2006) with some modifications. The samples of 100 berries were frozen until the analysis were carried out. The seeds were discarded before defreezing the samples at a temperature of 4 ºC. Subsequently each sample was homogenized in an Omni-Mixer (Sorvall, Labequip, Ontario, Canada) where 100 mg Na2S2O5 were added, centrifuged for 15 min at 4000 rpm and the liquid phase was recovered. The pellet was washed with pH=3.2 solution and centrifuged again. This operation was repeated twice. The liquid phases were then assembled up to 300 mL, adding 100 mg of a commercial pectolitic enzyme (Vinozym, Novo Nordisk Ferment Ltd, Dittingen) without glycosidase activity. After a period of six hours they were filtered using filtered discs (Whatman 589). 150 mL of these extracts were passed through a Sep Pack ® Cartridge C18 5 g (Waters, Milford, MA, USA) previously activated with 20 mL of methanol (Sigma–Aldrich Co, St Louis, MO, USA) and 50 mL of water. After treating each sample, the cartridge was washed with 50 mL of water and subsequently with 30 mL of dichloromethane. The bound compounds were eluted with 25 mL of methanol. Methanol was then eliminated under vacuum and the residue was solubilised in 5 mL of 0.1 M citrate-phosphate buffer (pH=5.0; 51.5% 0.2 M sodium phosphate and 48.5% 0.1 M citric acid). When analyzing red variety samples, 1 g of polyvinylpolypyrrolidone (PVPP; Sigma, St Louis, MO, USA) was added to remove the phenolic compounds. The glycosidically-bound fraction was hydrolyzed with 200 µL of a commercial glycosidase rich enzyme (Cytolase M102, Ferrari- s.r.l. Italy) at 40 ºC for 24 h. A known concentration of internal standard (1-heptanol at a final concentration of 100 µg L^{-1}; Fluka, Sigma–Aldrich Co.; St Louis, MO, USA; purity assay>99.0%) was added to the mixture containing the aglycons released by enzymatic hydrolysis at room temperature. The suspension was then centrifuged at 4000 rpm during 15 minutes to remove PVPP. The supernatant was then passed through a Sep Pack ® Cartridge C18 1 g previously activated with 5 mL of methanol and 10 mL of water. The free compounds were recovered with 12 mL of dichloromethane. After drying with anhydrous sodium sulphate, the obtained volume was evaporated at ambient temperature and pressure. After concentration the sample was ready for GC/GC-MS analysis.

Gas Chromatography-Mass Spectrometry conditions

The separation was achieved using an Innowax 19091-N (Agilent-J&W Scientific, Santa Clara, CA, USA) capillary column (30 m x 0.25 mm ID, 0.25 µm d.f.), using helium as the carrier gas at 70 kPa. The gas chromatograph was a Hewlett Packard 5890 series II connected to a selective detector MSD 5970 (Hewlett Packard, Wilmington, DE, USA). The injector temperature was 250 ºC and interface temperature was 230 ºC. The oven temperature program was set at 45 ºC for 2 min, then linearly increased, at a rate of 30 ºC min^{-1} to 60 ºC, 2 ºC min^{-1} to 160 ºC, and 3 ºC min^{-1} to 230 ºC. The final temperature was maintained for 13 min. The injection mode was splitless for 2 min. Acquisition mass ranged was from 28 to 300 u.m.a. and ionization energy was 70 eV. Volatile compounds were identified by comparing the retention time and mass spectra with those reported in the literature (di Stefano 1991; Cabrita et al. 2006). All mass spectra were also compared with those of the data system libraries (Wiley275 and libraries of mass spectra). Semi-quantitative data were obtained by the ratio of area of individual compounds versus internal standard area (1-heptanol).

Threshold determination and odour activity value (OAV)

The odour perception threshold is defined as the lowest concentration able to produce a sensation. In order to identify the most powerful aroma compounds of the studied grapevines and the most interesting varieties, the odour activity value (OAV) was used. This was calculated as the ratio between the concentration value of the compound and the perception threshold found in the literature (Table 2). Previous studies report that when the OAV is greater than 0.20 the compounds contribute to the aroma (Versini et al. 1994). Consistently with the literature, the compounds with OAV>0.20 were grouped in seven odorant series (floral, spicy, citric, fruity, phenolic, roasted and sweet; Table 2). The OAV of a series was calculated by summing up the individual OAV for all the compounds included in each odorant series. To allow comparisons of the aroma profiles across different grapevines the relative aroma values in each odorant series was calculated.

Table 2. Odour threshold (µg L^{-1}), odour description and odorant series (1, citric; 2, floral; 3, fruity; 4, phenolic; 5, roasted; 6, spicy; 7, sweet). Only the compounds with OAV>20% are shown

Aroma compound	Odour threshold (µg L^{-1})	Odour description	Odorant series
Benzaldehyde	300 (Darriet et al. 2002)	Almond, fragrant (Peinado et al. 2004), piney, fruity (Noble et al. 1980), roasted (Franco et al. 2004)	2, 5 (Franco et al. 2004)
Benzyl alcohol	620 (Latrasse 1991)	Fruity (Lorrain et al. 2006), floral (Fang and Qian 2006; Lorrain et al. 2006), roasted, toasted (Franco et al. 2004)	5 (Peinado et al. 2004)
2-phenylethanol	1500 (Zamuz et al. 2006)	Floral, fruity, grassy (Noble et al. 1980), rose, honey (Franco et al. 2004)	2 (Zamuz et al. 2006)
Eugenol	5 (Guth 1997)	Clove, balsamic, peper (Arroyo et al. 2009), cinnamon, wood (Moyano et al. 2002)	4, 6 (Moyano et al. 2002)
4-vinylguaicol	40 (Guth 1997)	Spicy, woody (Ugliano and Moio 2008), phenolic (Arroyo et al. 2009)	4 (Arroyo et al. 2009)
4-vinylphenol	180 (Boidron et al. 1988)	Spicy, phenolic, cypress, vanilla (Lorrain et al. 2006)	6 (Lorrain et al. 2006)
Linalool	15 (Guth 1997)	Citrus, floral, sweet, grape-like (Peinado et al. 2004)	2, 3, 7 (Peinado et al. 2004)
α-terpineol	80 (Zamuz et al. 2006)	Floral, lilac, sweet (Peinado et al. 2004)	2, 7 (Peinado et al. 2004)
Citronellol	18 (Ribéreau-Gayon et al. 2006)	Citronella (Ribéreau-Gayon et al. 2006), green, clove (Ferreira et al. 2001)	1, 2 (Zamuz et al. 2006)
Nerol	80 (Zamuz et al. 2006)	Floral (Ugliano and Moio 2008; Moyano et al. 2002), green (Moyano et al. 2002)	2 (Moyano et al. 2002)
Geraniol	30 (Guth 1997)	Rose (Ribéreau-Gayon et al. 2006), floral (Ugliano and Moio 2008)	2 (Ugliano and Moio 2008)

Statistical analysis

The aromatic compounds and varieties were analyzed using Stepwise Forward Discriminant Analysis (SFDA) and Principal Component Analysis (PCA). SFDA was used in order to differentiate white, rose and black varieties, and the origin of Beba samples. The PCA analysis allowed establishing a relationship between the different aroma compounds and the studied grapevines, as well as identifying the most important compounds in white, rose and red varieties. The correlations between different accessions of the same variety were evaluated using Pearson correlation analysis (r). The differences between OAV for each series and between varieties included and excluded from Quality Demarcations were checked by ANOVA followed by Tuckey´s HSD test to enable pairwise comparisons of means ($p<0.05$). All statistical analyses were performed using XLSTAT 2009 version.

Results

In this study, 51 glycosidic compounds from 21 wine varieties were identified and classified into four categories (alcohols, benzenes, terpenes and norisoprenoids; Appendix III: Tables 1, 2; Appendix III: Figures 1, 2). Total amount of glycosidic compounds varied from 947 (Fogoneu variety) to 2911 µg kg^{-1} (Sabaté variety). The compounds benzyl alcohol and 2-phenylethanol were the most abundant compounds for all the considered varieties. At the same time, all varieties presented high values of benzenic compounds, except Beba (E43 and O44 accessions), Beba roja and Giró (E31 accession) which instead presented high value of norisoprenoid compounds and Giró (E36 accession) which presented high value of terpenic compounds.

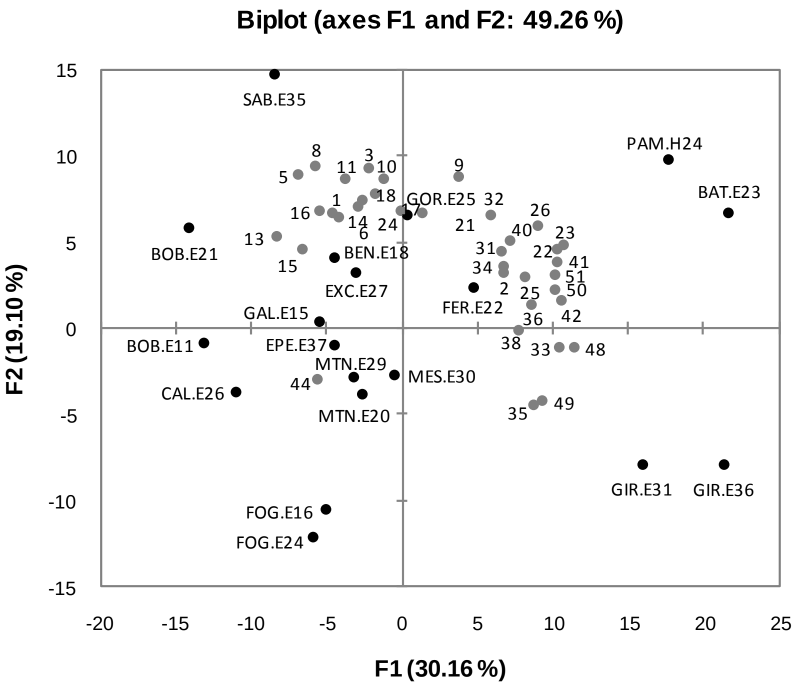
Glycosidic compounds characterization

Two PCA were done, one for each grape colour (one for red and other one for white and rose varieties). The first PCA axis for the red varieties (Figure 1a) explained the 30.2% of the total variance. The most important compounds in the first axis were hydroxy geraniol and diol (2,6-dimethyl-3,7-octadien-2,6-diol), accounting for 6.9% and 5.9%.of the total variance explained by this axis respectively. These alcohols were highly related with Batista, Pampolat girat and Giró varieties. The second axis explained 19.1% of the total variance and it was related with benzyl alcohol (7.3% of the variance), trans-2-hexenol (7.2%) and benzaldehyde (6.5%). Sabaté was related with benzyl alcohol compound showing a more than double value with respect to the rest of the analyzed varieties. Pampolat girat, Batista, Gorgollassa and Fernandella varieties were correlated to norisoprenoid and terpenic compounds. In addition, Pampolat girat was correlated to 3-oxo-α-ionol, displaying the highest concentration (234 µg kg^{-1}). Fogoneu, Callet, Manto Negro, Eperó de Gall, Bobal (accesion E21) and Mansés de Tibbus varieties obtained negative values on both axes. Giró was correlated to linalool and cis-8-hydroxy-linalool compounds, reaching the highest values (60 and more than 240 µg kg^{-1} respectively). The Cresol aroma exerted an influence on Bobal (E21 accession), Callet, Fogoneu and Galmete classification. Finally, Sabaté, Bobal (E21 accession), Beba negra, Excursach and Galmete showed a correlation with benzenic compounds.

As regards the differences among white and rose varieties (Figure 1b), the first PCA axis explained 40.8% of the total variation and was associated with diol (2,6-dimethyl-3,7-octadien-2,6-diol), explaining 4.5% of the variance, 4-vinylguaicol (4.3%), hexanol (4.2%) and blumenol C (4.2%). The second axis explained 23.5% of the variance being mainly related with 3-oxo-α-ionol (6.9%), hydroxy citronellol (6.2%) that separated Argamusa, Pensal Blanca and Quigat varieties and cis-8-hydroxy-linalool (5.4%). All the Beba accessions were linked with norisoprenoid and terpenic compounds, especially with 3-oxo-α-ionol, which attains the highest value (>166 µg kg^{-1}) and with cis-8-hydroxy-linalool (>60 µg kg^{-1}). Quigat was particularly related to methyl syringate, this being the only white variety presenting this compound. Argamusa was related with trans-2-

hexenol, dihydroconiferyl alcohol and endiol. Viñaté showed strong relation with benzaldehyde (140 µg kg^{-1}).

a)



b)

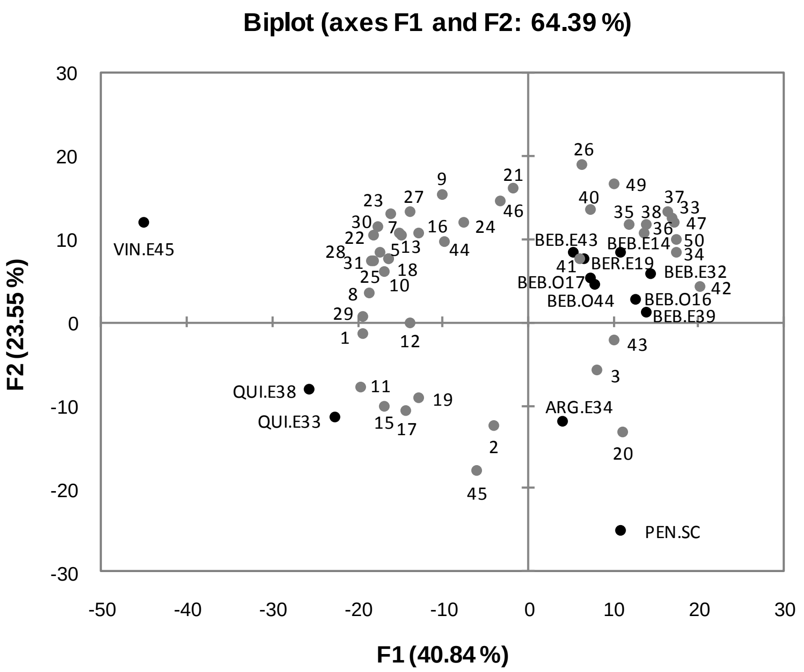


Figure 1. Principal Component Analysis plots for PCA1 and PCA2 of the aroma volatile compounds used for (a) red and (b) rose and white grapevines. Symbols: black circles (grapevine varieties) and grey circles (aroma from glycosidic precursors; keys in Appendix III: Tables 1, 2). The averages of the three replicate samples were used for the analysis

Odour active value (OAV)

Six benzenic compounds (benzaldehyde, benzyl alcohol, 2-phenylethanol, eugenol, 4-vinylguaicol, 4-vinylphenol) and five terpenic compounds (linalool, α-terpineol, citronellol, nerol, geraniol) displayed an OAV higher than 0.20 (Appendix III: Tables 3, 4). All the odorant series showed statistically significant differences (p<0.001) in both red and white varieties. Floral, spicy and phenolic were the most important odorant series in all the studied cultivars (Figures 2, 3).

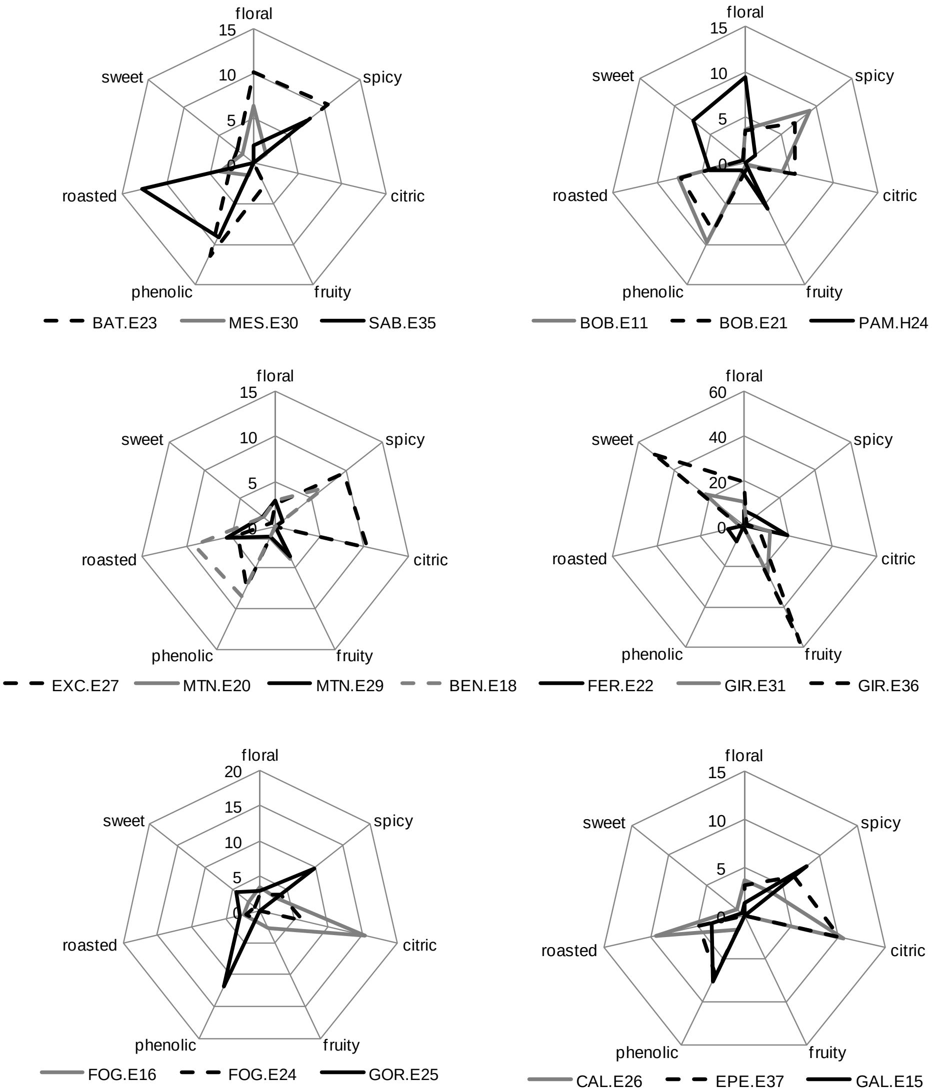


Figure 2. Mean Odour Activity Values (OAV), in relative unities, for odorant series in red varieties. All the series between cultivars were significantly different (p <0.05). Keys in Table 1

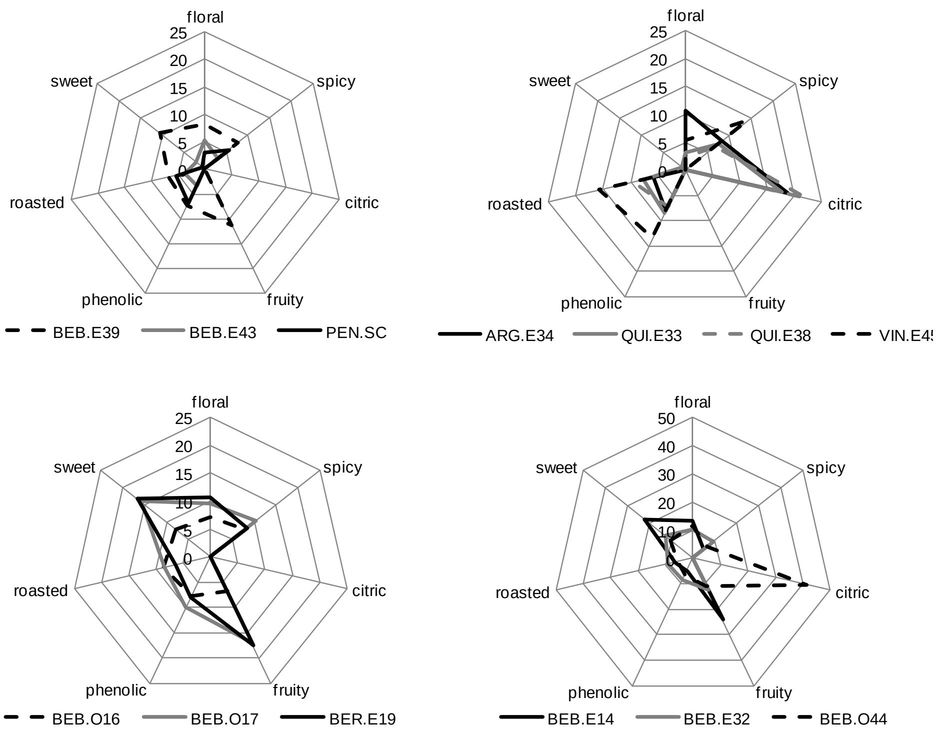


Figure3. Mean Odour Activity Values (OAV), in relative unities, for odorant series in white varieties. All the series between cultivars were significantly different (*p*<0.05). Keys in Table 1

Among red varieties, Giró (E36 accession) was the most important in floral, fruity and sweet series. Batista was the spiciest variety and, together with Gorgollassa, the most phenolic one. Sabaté scored the highest in the roasted series. Among rose and white varieties, Beba (E14 accession) presented the highest OAV in the floral, fruity and sweet series, and Viñaté was the most important variety in spicy, phenolic and roasted series.

In the cases where several accessions of the same variety were studied, only Beba and Giró varieties displayed statistically significant differences (*p*<0.001). As a matter of fact, three accession of Beba variety presented the highest OAV, E14 in floral, citric and sweet series; O44 in citric series and E32 in roasted series. As a far as Giró is concerned (E36 accession) presented the highest OAV in floral, fruity and sweet series, while E31 accession presented the highest OAV in citric series.

A comparison between varieties included in a Demarcation of Quality and those excluded highlighted statistically differences in the OAV of red varieties, in the floral and phenolic series (*p*<0.001). In both cases, the varieties not included in a Quality Demarcation were the richest ones. White varieties presented statistically significant differences (*p*<0.001) in several series. In this case, varieties included in a Quality Demarcation turned out to be the richest ones in the fruity and sweet series. However, the varieties not belonging to any Quality Demarcation presented the highest values in the spicy, phenolic and roasted series.

Role of the berry colour

The within-class covariance matrices reported differences between berry colour (SFDA analysis, $p<0.001$). All the studied samples were classified correctly except one replicate of Beba (O16 accession), which changed from the white to the rose category. The percentage of correct classification for the 96 samples analyzed was 98.96% (Figure 4). The most suitable variables for this classification were two alcohols, three benzenic compounds and four terpenes. As regards the alcohols, hexanol turned out to be higher in red varieties ($p<0.001$; 65.55 *vs* 33.93, 36.76 µg kg^{-1} per red, rose and white samples respectively) and tirosol was present exclusively in red varieties ($p=0.026$; 4.42 µg kg^{-1}). As regards the benzenic compounds, 2-phenylethanol was higher in rose samples ($p<0.001$; 257.77 *vs* 186.80, 238.97 µg kg^{-1} per rose, red and white samples respectively), 4-vinylphenol was higher in red samples ($p<0.001$; 91.36 *vs* 10.14 and 14.25 µg kg^{-1} per red, rose and white samples respectively) and methyl vanillate appeared to be higher in red samples than in white samples ($p<0.001$; 22.20 *vs* 3.35 µg kg^{-1} respectively). Finally, the four relevant terpenes were trans-furan linalool oxide, higher in rose samples ($p=0.033$; 16.21 *vs* 8.35 and 7.05 µg kg^{-1} per rose, red and white samples respectively), α-terpineol was higher in red followed by rose and white varieties ($p=0.016$; 11.06, 7.21 and 4.76 µg kg^{-1} respectively), endiol, higher in white samples than in red varieties ($p=0.002$; 2.32 µg kg^{-1} *vs* 0.74 µg kg^{-1} respectively), and p-menthene-7,8-diol, higher in red samples ($p=0.026$; 28.12 *vs* 16.78, 11.64 µg kg^{-1} per red, rose and white samples respectively).

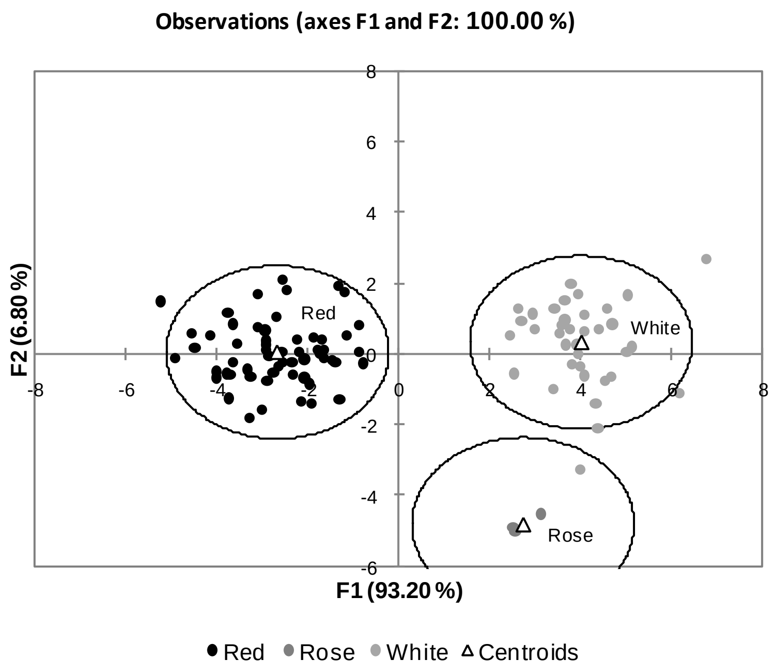


Figure 4. Discriminant Analysis (SFDA) of aroma from glycosidic precursors from grapevines (*Vitis vinifera* L.) grouping according grape colour. Symbols: triangles (centroids of each group) and circles (grapevine varieties). Three replicates per sample were used in the analysis

Role of the clone

The correlation between accessions of the same variety was higher than 0.90, with the notable exception of Beba variety (r=0.81). All accessions of Bobal, Giró, Manto Negro and Quigat varieties presented r>0.97. Nonetheless, they showed slight differences between accessions of the same variety (Appendix III: Tables 1, 2).

Role of the origin of the Beba accessions

If we consider the origin of each Beba accession (PM, GI and LE), the result of the SFDA method indicated that the within-class covariance matrices were different (p<0.001). All the varieties were classified correctly (Figure 5). The variables that showed significant differences were one benzenic compound, namely homovanillyl alcohol, higher in samples from PM (p=0.002; 26.22 *vs* 10.57, 13.82 µg kg^{-1}; PM, GI, LE, respectively) and four terpenes, namely α-terpineol, higher in samples from PM (p=0.036; 8.46 *vs* 4.53, 5.93 µg kg^{-1}; PM, GI, LE respectively), trans-pyran linalool oxide, higher in PM samples (p=0.004; 22.03 *vs* 14.68, 15.89 µg kg^{-1}; PM, LE, GI, respectively), citronellol, exclusively present in samples from LE (p=0.078; 1.92 µg kg^{-1}) and diol (2,6-dimethyl-3,7-octadien-2,6-diol), lower in samples from PM (p=0.011; 7.55 *vs* 9.72, 10.71 µg kg^{-1}; PM. LE, GI, respectively).

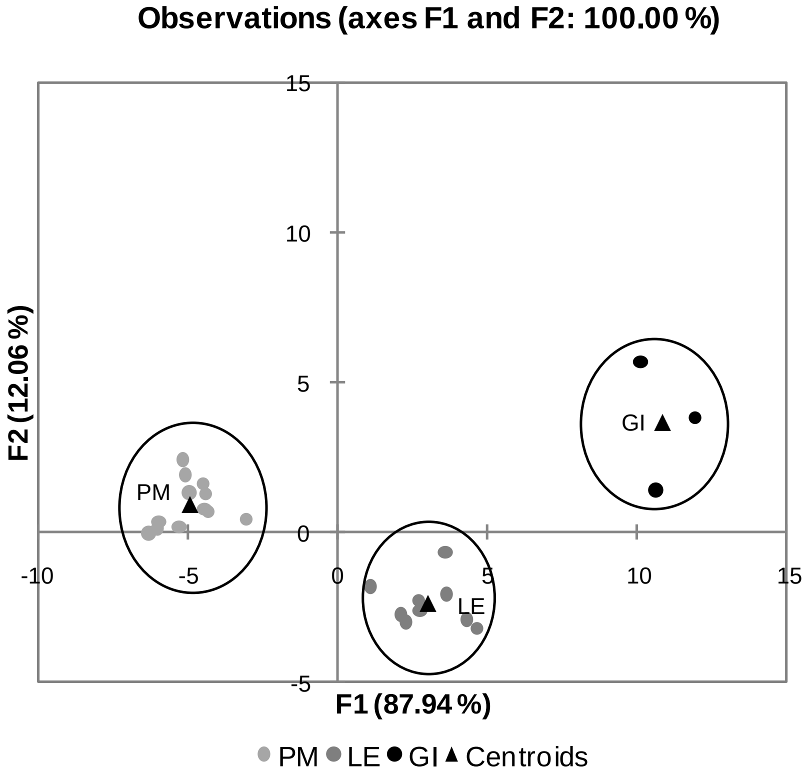


Figure 5. Discriminant Analysis (SFDA) of aroma from glycosidic precursors from Beba accessions grouping according geographical origin: Palma de Mallorca (PM), Levante area (LE) and Girona (GI) Symbols: triangles (centroids of each group) and circles (grapevine varieties). Three replicates per sample were used in the analysis

Discussion

Glycosidic compounds characterization

The results achieved in this study for Callet and Manto Negro were consistent with previous descriptions, even though different techniques have been used and the varieties were planted in a different location (Forcen et al. 1993). Some aromatic compounds, such as benzyl alcohol, benzaldehyde (Rosillo et al. 1999; Bueno et al. 2003), 2-phenylethanol (Bueno et al. 2003; Genovés et al. 2005), or 1-hexanol (Genovés et al. 2005) have been found to be a key factor in the characterization of grapevines. All of these compounds were important glycosidics in this study.

3-oxo-α-ionol was relevant for the classification of both white and red varieties; however other authors found the same quantity of 3-oxo-α-ionol in other 13 different varieties (Rosillo et al. 1999). The presence of this compound could be related to biological activity (Stuart and Coke 1975), being one of the most important C13-norisoprenoids in grapevine (Baumes et al. 2002). This is the case for Pampolat girat and Beba varieties. Vinylphenol was always accompanied by vinylguaicol and it is well-known that these compounds are products from of the breakdown of *p*-coumaric and ferulic acid that are present in grapes and musts (Chatonnet et al. 1995).

Several red varieties were characterized by cresol, which presents a high odorant impact acknowledged by neurological studies (Ryan et al. 2008). On the other hand, methyl syringate, a shikimate derivative, was present in all red varieties and in only white variety namely Quigat, although the existence of this compound in other white cultivar has been previously recorded (Cabaroglu 2002). Benzaldehyde commonly associated with presence of *Botritis cinerea* (Delfini 1991), was present in Viñaté variety with the highest value (140 µg kg^{-1}), however no other compound associated with *Botritis cinerea,* for instance 1-octen-3-ol, has been found (Pallotta et al. 1998). Benzaldehyde has also been reported with significant values in other Spanish varieties (Bueno et al. 2003; Genovés et al. 2005), therefore we could consider a high amount of this compound to be a characteristic of this variety. Blumenol C played an important role in the characterization of white and rose varieties. This compound is a derivate of Blumenin (Maier et al. 1995), which has been proved to possess antifungal properties in barley and wheat (Fester et al. 1999). As a consequence, this compound could have a function in resistance mechanisms. The mentioned aroma compounds from glycosidic precursors are important compounds for the characterization of the studied cultivars.

Odour active value (OAV)

The aroma compounds impact selection is based on three factors: the perception threshold, the compounds concentration (Lorrain et al. 2006), and the criterion to select the OAV. Most studies consider the OAV to be higher than one (Guth 1997; Rocha et al. 2004), or 0.20 (Versini et al. 1994). Aromatic compounds presenting an OAV lower than one contribute to the aroma profile in neutral varieties (Escudero et al. 2004; Loscos et al. 2007), being the key compounds to define the aroma in a beverage (Ryan et al. 2008). Moreover, the attribution of an odorant series to volatile compounds is a validated procedure and represents the first step to approach the wine aroma profile (Ferreira et al. 2000; Franco et al. 2004). This procedure allows interpreting the outcomes of the chemical analysis via a sensory perception analysis to obtain an aroma description. The results found in this study corroborate this hypothesis, since the compounds with higher odour active value have been proved to be odour-active compounds in musts (Franco et al. 2004) and wines (Rocha et al. 2004; Ugliano and Moio 2008).

When several accessions of Beba and Giró varieties were studied, differences among their aromatic profiles were found. It seems possible to choose clones with higher aroma potential to obtain wines with different aroma profile (Marais and Rapp 1991; Botelho et al. 2010).

Comparing the aromatic profile series between the varieties included and excluded from Quality Demarcations, we have demonstrated that excluded varieties had greater profiles in several odorant series with respect those included in Quality Demarcations. An example of this is the Gorgollassa variety, whose wines obtained several prizes towards the end of the XIX century (Anonymous 1878), nowadays this variety is excluded from all Quality Demarcations.

Role of the berry colour

The differences between red and white grapevines are caused mainly by a mutation on two genes (Walker et al. 2007). However, the origin of most of aroma compounds in plants is unknown, despite of the last research done in this field among the last years (Dudareva et al. 2006; Chen et al. 2011). Due to the different aroma composition in grape cultivars the wine made from white and red varieties can be clearly differentiated, since the aromatic profile depends on the cultivars (Ferreira et al. 2000; Esti and Tamborra 2006) and the relationship between grape and wines aroma compounds has been demonstrated (Mulet et al. 1992; Ugliano and Moio 2008).

The influence of the berry colour in the aroma characterization of Spanish varieties has been also studied by different authors (Muñoz-Organero and Ortiz 1997; Rosillo 1999). The results of this study are consistent with the reviewed literature, since the varieties were grouped and differentiated according to the berry colour. However, volatile compounds were different from those found in other studies (Rosillo et al. 1999), in which the grapevines did not easily fall into the identified groups (Muñoz-Organero and Ortiz 1997; Rosillo et al. 1999). This could be explained considering the larger number of grapevines included in this study with respect to other studies. Our results showed that two alcohols, three benzenic compounds and four terpenes were the main volatile compounds for the differentiation of white and red varieties. The hexanol compound reached higher values in red varieties, which is consistent with previous studies (Rosillo et al. 1999). This compound has a grape origin (Ferreira et al. 2000) and it is scarcely affected by the fermentation process (Bueno et al. 2003; Esti and Tamborra 2006). As regards the differences among benzenic compounds between white and rose varieties, it could be suggested a link between the biosynthetic pathway of benzene compounds and the shikimic acid pathway that leads to the synthesis of polyphenols, having them a point of contact represented by the phenylalanine (Haslam 1998; Dudareva et al. 2006). The synthesis of terpenic compounds has been appointed to be related to a genetic component (Duchêne et al. 2009a; Chen et al. 2011). Therefore, the genetic differentiation between red and white cultivar might be also related to the different grape aroma compound found in white and red varieties.

Role of the clone and samples origin

Differences between grapevine clones based on aroma compounds has been appointed by other authors (Marais and Rapp 1991; Duchêne et al. 2009b; Botelho et al. 2010), as well as the influences of the geographic origin over the aroma compounds in must (Marais and Rapp 1991; Mulet et al. 1992; Zamuz and Vilanova 2006). The clones of Beba variety differed as far as their glycosidic compounds were concerned, despite the fact that all varieties were planted in the same location, the quality and value of the glycosidic compounds was statistically different depending on the origin of Beba accessions, which could be Peninsular (Girona and Levante area) or island origin (Balearic Islands). Several benzenic or terpenic compounds have proved to be a useful in classifying must geographic origin, for instance α-terpineol, one of the most odoriferous monoterpene alcohols not affected by the winemaking procedure (Esti and Tamborra 2006), or diol (2,6-dimethyl-3,7-octadien-2,6-diol) an aroma precursor (Bayone et al. 2003). These terpenic compounds were crucial for the geographic grouping of Beba accessions. The synthesis of some terpenic compounds has been appointed to have a genetic component (Duchêne et al. 2009a; Duchêne et al. 2009b). The Beba variety, an ancient variety recorded in the Balearic Islands since 1730, in Levante area since 1791, or in Girona since 1877 (Anonymous 1878), has been conserved at the *Vitis* Germplasm Bank "Finca El Encín" since the beginning of the XX century. As a result, the Beba variety could have adapted to local environmental conditions, improving the synthesis of the aroma metabolic route with a cumulative effect over time.

Conclusions

This report is the first study addressing the potential aroma characterization of several Spanish minor varieties. Differences in the aroma profile between varieties included and excluded from Quality Demarcations have been assessed in several odorant series. Minor varieties excluded from Quality Demarcations appear to be more aromatic and, as a consequence, Argamusa, Gorgollassa and Pampolat girat varieties could be taken into consideration for the development of new wine market strategies, which could also play an important role in the conservation of these cultivars.

Glycosidic compounds were crucial to differentiate white, rose and red varieties. Significant differences between clones of the same variety have been identified as well as differences in benzenic and terpenic compounds based on the origin of the Beba variety. The results suggest that cultivar, berry colour, and cultivar adaptations to location and environmental conditions play an important role in the metabolic pathways to obtain the aromatic compounds. These metabolic pathways seem to have a genetic component.

However, after this study which has permitted to individualize the more significant compounds that characterize the potential aroma of those varieties, other criteria might be analysed. Agronomic characterization and further investigations, such as GC–olfactometric studies or quantitative data, are needed to verify the impact of the odour-active compounds identified in this analysis.

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Chapter 7

We will meet at 11 am

Sensory characterization and factors influencing quality of wines made from 18 minor varieties (*Vitis vinifera* L.)

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Abstract

The oenological market is alive, always eager for new varieties to satisfy the consumer´s demand. Thus, the minor or autochthonous wine varieties might be strong candidates to fill this gap. However the potential of most of these wine varieties is unknown. The quality of wine is difficult to assay, nevertheless quantitative and descriptive analysis are the most used methods in wine sensory characterization. The aim of this study was to characterize wines made from local cultivars using sensorial description. Wines including red, white and rose, made from 18 minor varieties in two vintages were analysed. Analysis of Variance, Principal Component Analyses and Partial Least Squares were used to track the influence of vintage, the inclusion of variety in Denomination of Origin (DO) and agronomical parameters over the sensorial attributes scored by 21 expert wine tasters. Expert´s preference map was also performed to identify the oenological possibilities of these wines.

This study highlights that the used questionnaire and the experts were efficient for the tasted wines. The effect of vintage was more important than the Denomination of Origin over both chemical and sensory characterization. Sensorial analysis conducted on wines demonstrated significant correlation between sensory attributes and agronomical parameters. Vegetative and productive agronomical parameters influenced inversely over aroma and taste scores by experts. The management of these parameters would improve the quality of wines. This study also highlights the wine market possibilities of minor varieties which have been accepted by wine experts. The varieties located in the best position of the expert preference map should be considered by technicians due to their quality, sometimes even better mapped than the wines made from varieties allowed in Spanish DOs. This information would gain a more interest of minor or autochthonous wine varieties, being the key for their preservation.

Key words: expert preference, local cultivars, sensory analysis, vintage, wine quality

Introduction

Spain is an important viticulture area in the world, which is divided on 73 Designations of Origin (DO) and included 250 different cultivars in its national grapevine catalogue, being the fourth country in grapevine diversity in the European Union behind Italy, Portugal and France (Lacombe et al. 2011). Despite of the high number of autochthonous cultivars the international varieties are widely spread and their cultivation are allowed in most of the Spanish DO. In contrast, most of the minor varieties that could be called “autochthonous” are not included in any DO in spite of some of them (e.g. Gorgollassa) presents a great oenological potential (Chapter 6; Gutierrez Afonso et al. 1998). DOs play an important role in food and wine marketing strategies (Douglas et al. 2001; Skuras and Vakrou 2002), since they are based not only in the geographic area but also in wines quality and originality. Nowadays, DOs are looking for wine varieties (*Vitis vinifera* L) link to the site (“authoctonous”), which could provide original and high quality wines, with the aim to increase market possibilities (Koussissi et al. 2008; Bertuccioli 2010). The use of minor varieties could be an excellent option to satisfy DO requirements.

The quality of the wines depends on grape variety, vintage and soil-type (Maitre et al. 2010), and bedside the quality of wine is difficult to assay (Charters and Pettigrew 2007), quantitative and descriptive analysis are the most used methods in wine sensory characterization (Murray et al. 2001; Perrin et al. 2007). The descriptive analysis of the commercial wines made with international grape cultivars have been largely studied as Riesling (Fischer et at 1999; Douglas et al. 2001), Sauvignon blanc (Parr et al. 2007; Lund et al. 2009), Chardonnay (Lee and Noble 2006), Cabernet-Sauvignon (Tao et al. 2009) or Pinot noir (Girard et al. 2001); studies related to this topic are available in Spanish commercial wines (de la Presa-Owens and Noble 1995; Vilanova et al. 2008; Rodríguez-Nogales et al. 2009; Muñoz-González et al. 2011). However, descriptive analysis of the not commercial wines done with local or autochthonous grape varieties are not been very usual (Gutierrez Afonso et al. 1998) despite of the great interest that this varieties are arousing nowadays, since oenology production is a dynamic process of that need to be always adapted to changes and demands on wine market (Bertuccioli 2010).

The aim of this study was to characterize wines made with local cultivars using sensorial description. Thus, wines including red, white and rose, made from 18 minor varieties have been studied in two vintages. The effect of vintage and varieties included or not in DO over the chemical analysis as well as over the wine sensorial descriptors was analyzed. The correlations between chemical analysis and agronomical variables and sensory characterization were also considered. Expert´s preference map was performed to identify the oenological possibilities of these wines made from minor varieties, providing a valuable tool to make out the potential of this wines in future wine market. It hopes that this information would lead to improve the quality of the wines and help to gain a more interest of autochthonous varieties.

Material and Methods

Plant material selection, field conditions and agronomical parameters

The cultivars (*Vitis vinifera* L.) were selected according to two criteria (1) their geographic origin, all of them were collected in the Balearic Islands and were linked to the site (García-Muñoz et al. 2011), and (2) varieties allowed or not in Spanish DO were selected (Table 1).

Table 1. List of the wines analyzed; conserved at the *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain). N: Number of Spanish Designation of Origin where the variety is allowed; - Minor varieties non included in a Designation of Origin. All the wines were mono-varietal (from 100 % of the same variety) except Fogoneu and Eperó de Gall wine which was done from (63 and 37% respectively from each variety)

Varieties used	Berry colour	Winemaking protocol	Studied vintage	N
Batista	Black	Red	2006, 2007	-
Bobal	Black	Red	2006, 2007	6
Callet	Black	Red	2006, 2007	2
Eperó de gall	Black	Red	2006, 2007	-
Excursach	Black	Red	2006, 2007	-
Fogoneu	Black	Red	2006, 2007	1
Mandón	Black	Red	2007	-
Gorgollassa	Black	Red	2006, 2007	-
Manto Negro	Black	Red	2006, 2007	2
Pampolat girat	Black	Red	2006, 2007	-
Valenci Tinto	Black	Red	2006	-
Eperó and Fogoneu	Black	Red	2006	
Beba roja	Rose	Rose	2006, 2007	-
Giró	Black	Rose	2007	-
Sabaté	Black	Rose	2007	-
Mansés de Tibbus	Black	Rose	2007	-
Beba	Green-yellow	White	2006, 2007	1
Pensal Blanca	Green-yellow	White	2006, 2007	2
Quigat	Green-yellow	White	2006, 2007	-

All the varieties were cultivated at the *Vitis* Germplasm Bank “Finca El Encín” (IMIDRA, Alcalá de Henares, Spain). Plants were almost five years old and were grown under the same field conditions. All of them were grafted onto 110R and were irrigated until veraison. It had a unilateral cordon trellis system and eight buds per vine were left in winter pruning. The distance between vines was 0.9 m and the one within rows was 2.5 m. A set of 11 agronomical parameters were studied in vine plants: grape harvested per vine (kg), number of bunches per vine, number of bunches per shoot, bunch weight, number of berries per bunch, berry weight and must yield were measured in harvest, whereas number of shoots per vine, Kg of pruning wood per kg of grape, woody shoots weight per vine and woody shoots weight were measured during vines dormancy (OIV 2001; Table 2).

Table 2 Agronomical parameters of the 18 studied varieties. Data are the media of the 2006 and 2007 vintages. Missing data are scored as "md"

Variety	Kg grape/ Vine	Number of bunches/Vine	Number of bunches/Shoot	Kg pruning wood/kg grape	Total woody shoots weight/Vine	Woody shoots weight (g)	Number of woody shoot/Vine	Bunch weight (g)	Number of berries/Bunch	Berry weight (g)	Must yield (%)
Batista	0,60	7,53	0,84	0,42	0,22	27,60	7,89	170,78	121,17	1,67	30,06
Beba blanca	1,21	5,81	0,71	0,46	0,41	52,75	7,91	222,07	97,00	2,91	24,28
Valenci Tinto	0,48	4,41	0,49	2,56	0,43	56,33	7,48	148,02	75,83	3,38	28,20
Beba roja	0,91	5,65	0,89	0,53	0,37	55,23	6,61	285,70	124,67	2,97	23,60
Bobal	0,75	3,58	0,43	0,37	0,23	23,24	9,80	233,77	134,67	2,41	31,07
Callet	1,16	6,98	0,85	0,15	0,16	17,64	8,59	379,61	199,33	2,39	28,54
Eperó de Gall	0,74	6,86	0,88	0,85	0,43	53,60	8,00	299,66	198,50	2,12	28,26
Excursach	1,15	8,54	1,09	0,98	0,27	30,83	8,25	293,12	180,33	1,74	24,52
Fogoneu	1,10	9,29	1,08	0,51	0,26	21,68	8,77	276,76	158,17	2,34	25,10
Mandón	md	md	md	md	md	md	md	366,70	251,83	1,79	27,22
Giró	1,93	9,79	1,13	0,21	0,32	36,79	8,51	298,76	173,08	2,20	31,06
Gorgollassa	0,61	6,83	0,85	0,82	0,31	38,95	7,89	152,90	80,67	2,24	27,35
Mansés de Tibbus	1,78	8,35	0,98	0,39	0,50	59,66	8,52	328,25	195,33	2,32	31,01
Manto Negro	1,10	7,04	0,87	0,26	0,27	33,65	7,89	300,50	180,33	2,05	22,79
Pampolat girat	0,81	6,82	0,86	0,60	0,30	35,51	8,43	223,92	153,67	1,69	29,72
Pensal Blanca	2,36	9,12	1,26	0,19	0,31	35,29	8,26	486,98	223,75	2,52	32,46
Quigat	1,83	9,46	1,32	0,14	0,16	21,76	7,65	378,15	181,50	2,70	21,23
Sabaté	2,14	9,02	1,07	0,15	0,22	27,34	8,15	403,77	316,17	1,68	33,53

Winemaking protocol

In this study we analyzed 32 wines including red, white and rose, made from 18 different varieties. The wines were elaborated in two vintages, 15 wines were made in 2006, and 17 wines in 2007. Depending of the available quantity of grape harvested, 13 of them were common in the two vintages (Table 1). All wines were mono-varietal, made from 100% of the same specified variety except a wine made from a blend of Fogoneu and Eperó de Gall varieties in 2006 (63 and 37% respectively; Table 1). The grapes were picked by hand, in excellent sanitary conditions, at commercial maturation during 2006 and 2007 vintages. The fermentation was carried out following traditional winemaking methods. Grapes were destemmed and slightly crushed using an electric crusher (CME Construzioni Machine Enologiche, Italia). The musts were treated with sulphur dioxide liquid (20, 25 and 25 mg L^{-1} SO_2 equivalent for red, white and rose respectively) and macerated 24h approximately with pectolitic enzyme (Vinozym, Novo Nordisk Ferment Ltd, Dittingen) at 10ºC. The white and rose musts were pressed using a manual press and then they were maintained at 10ºC during 12h, after that the musts were racked.

All must were inoculated with yeast *Saccharomyces cerevisiae*. The yeast Fermol PB 2033, Fermol Reims Champagne and Fermol Grand Rouge nature (Pascal Biotech, AEB Group, France) were used for red, white and rose varieties winemaking respectively. They were prepared according to the manufacturer´s recommendation. Depending of the grape harvest available, all fermentation were carried out in glass container for must volume less than 17L, and in stainless steel tank “always full” for more than 17L. The fermentation temperature was maintained between 25 and 30°C for red wines and between 15-20ºC for white and rose wines in a dark air-conditioned room. In red wines, fermentation caps were punched down twice a day each 12h. In all wines, fermentations were monitored for temperature and density upon completion of the alcoholic fermentations indicated by constant density. When the fermentations red wines finished, the wines were manually racked off and pressed in a manual press. Then they were transferred to glass recipients according to its final obtained volume. Red wines were inoculated with *Oenococus oeni* lactic acid bacteria (Vinifer, Agrovin, Spain) to induce malolactic fermentation. After that, the wines were racked.

The red wines were clarified with 10 g hL^{-1}, of commercial egg albumin and white and rose wines with 10 g hL^{-1} of bentonite. The wines were stored for 7 days at 8ºC in a dark room to allow its cold stabilisation. Afterwards, the wines were racked again. Prior to the final bottling, sulphur dioxide was adjusted to 40 mg L^{-1}. The wines were filtered through stainless steel filter holders (INLET YY3014236, Millipore Bedford, MA, USA) using N_2 as carried gas and 0.45µm filter disc (Millipore, Bedford, MA, USA). Then they were bottled in 750 ml of capacity, labelled and horizontally stored in a conditioned room kept at 10-12 ºC and 70% air humidity until sensory analysis, which was carried out around 4 months after bottling in each vintage. At the same time, for each wine, a bottle was set apart for chemical analysis, which was conducted 5 months after fermentation was completed.

Chemical analysis

Alcohol content (% vol), relative density, dry extract, volatile acidity, total acidity (expressed in g L^{-1} TH_2), pH, free SO_2, reducing sugar, total phenolic index, colour intensity, malic acid and tonality were determined according to European Union Commission Regulation method (RCEE 2676 1990; Table 3). All analyses were run three times and were done in the confirmed IMIDRA laboratory (UNE-EN ISO/IEC 17025, 2000).

Table 3 Chemical analysis of the 32 studies wines. Missing data (md)

Variety	Vintage	Alcohol content (%)	Relative density	Dry extract	Volatile acidity	Total acidity (gL^{-1} TH_2)	pH	Free SO_2	Reducing sugar	Total phenolic index	Colour intensity	Malic acid	Tonality
Beba blanca	2006	13.2	1.0005	19.6	0.67	5.5	3.46	10	3.3	8.51	0.160	1.7	md
Pensal Blanca	2006	12.0	0.9904	15.8	0.37	6.2	3.02	12	2.2	8.12	0.131	0.6	md
Quigat	2006	8.9	0.9921	10.7	0.28	6.4	3.17	13	2.4	8.19	0.163	1.1	md
Beba roja	2006	11.5	0.9922	18.8	0.20	6.20	3.35	21	2.3	8.97	0.187	1.9	md
Batista	2006	13.2	0.9928	22.9	0.55	4.5	3.71	30	2.3	29.88	1.606	0.1	md
Valenci Tinto	2006	14.4	0.9923	27.9	0.41	6.3	3.69	28	1.7	34.53	2.055	1.5	md
Bobal	2006	13.5	0.9920	24.8	0.30	5.6	3.56	32	1.5	40.36	10.332	1.0	md
Callet	2006	15.1	0.9916	30.7	0.33	5.4	3.91	30	1.6	58.32	4.520	2.3	md
Eperó de Gall	2006	17.0	0.9931	37.4	0.84	6.5	3.79	28	1.9	42.10	3.739	2.5	md
Eperó and Fogoneu	2006	13.1	0.9927	25.3	0.57	5.0	3.72	30	2.2	40.43	4.360	0.1	md
Excursach	2006	13.3	0.9924	25.0	0.22	6.3	3.41	27	1.9	42.76	8.706	1.1	md
Fogoneu	2006	12.8	0.9926	24.2	0.37	6.1	3.50	21	2.0	40.58	7.311	0.1	md
Gorgollassa	2006	14.4	0.9912	25.0	0.29	6.2	3.51	28	1.3	42.31	4.011	1.2	md
Manto Negro	2006	16.3	0.9909	27.1	0.36	5.8	3.62	37	2.1	42.25	3.570	1.8	md
Pampolat girat	2006	12.2	0.9932	28.4	0.57	4.3	3.92	25	1.4	57.67	3.850	0.0	md

Variety	Vintage	Alcohol content (%)	Relative density	Dry extract	Volatile acidity	Total acidity (g L^{-1} TH_2)	pH	Free SO_2	Reducing sugar	Total phenolic index	Colour intensity	Malic acid	Tonality
Beba blanca	2007	13.3	0.9909	21.1	0.55	5.5	3.70	57	2.9	8.02	0.115	2.8	5.197
Pensal Blanca	2007	12.5	0.9913	19.6	0.42	4.9	3.70	23	3.2	11.07	0.097	2.1	4.596
Quigat	2007	12.5	0.9912	19.6	0.24	6.0	3.29	22	2.2	7.91	0.103	2.1	4.404
Beba roja	2007	11.3	0.9931	20.9	0.26	5.7	3.41	22	2.4	6.84	0.144	2.1	3.015
Giró	2007	12.5	0.9939	21.6	0.36	5.3	3.38	30	3.2	10.55	0.469	1.8	1.180
Mansés de Tibbus	2007	14.1	0.9891	18.8	0.48	4.5	3.69	35	1.6	15.19	0.723	2.2	1.203
Sabaté	2007	14.4	0.9891	19.3	0.31	5.4	3.61	40	1.7	13.09	0.709	2.9	1.069
Batista	2007	10.1	0.9949	22.2	0.22	5.3	3.72	39	1.8	40.00	1.337	2.8	1.304
Bobal	2007	12.4	0.9935	25.3	0.22	5.2	3.76	33	1.2	40.40	6.069	0.4	0.777
Callet	2007	13.4	0.9926	25.5	0.45	3.4	4.16	42	1.2	32.22	2.608	0.1	1.352
Eperó de Gall	2007	14.4	0.9925	28.6	0.27	6.7	3.78	28	1.3	31.19	2.697	3.6	1.238
Excursach	2007	14.9	0.9921	28.9	0.29	6.1	3.69	42	1.6	45.87	7.335	2.1	0.787
Fogoneu	2007	12.8	0.9938	26.8	0.43	5.3	3.87	37	1.6	39.62	4.716	0.1	0.927
Mandón	2007	13.8	0.9930	27.9	0.30	6.2	3.64	29	1.3	31.44	3.447	2.4	0.995
Gorgollassa	2007	13.8	0.9936	3.6	0.24	6.2	3.78	28	1.0	31.80	4.586	2.8	0.991
Manto Negro	2007	14.5	0.9917	26.6	0.39	4.1	4.04	29	1.0	28.17	2.324	0.0	1.298
Pampolat girat	2007	15.0	0.9931	31.5	0.38	4.6	4.00	18	1.2	38.37	3.817	0.0	1.284

Sensory descriptive analysis

A panel of 21 volunteer tasters from Oenological and Viticulture department belonging to IMIDRA staff participated in the study (14 male and 7 female). All tasters had previously participated in wine sensory descriptive analysis studies, so they had great wine-judging experience, however one of them who was not adjusted for all sensory descriptors was rejected of the panel (data do not show). Nine of them (6 male and 3 female; mean year=39, range=30-49) who tasted all wines were chosen to analyze the wines, however the final mark from the 20 volunteers tasters were used to performance the expert´s internal preference map.

All sessions were conducted at the IMIDRA tasting room in individual booths. The tasting room was normalized (UNE-EN ISO/IEC 17025, 2000), room temperature of 20–22ºC, 60–70% relative humidity. Each sample (30 mL) was coded by three random digits, covered with Petri dishes and served in standard wine tasting glasses in accordance with the International Organization for Standardization (Norme ISO 3591 1977). The tasting sessions started at 11 am and took approximately 1h. Six wines at 17 ºC, presented in random order (Norme ISO 3591 1977), were tasted per session, so three sessions were needed each year. Tasters were asked to first rate the visual attributes of the wine, followed by odour and oral evaluations, and they were instructed to expectorate the samples as well as to rinse with mineral water and unsalted crackers to eliminate residual sensations between samples. Wines were tasted only once since a low quantity wine was obtained.

The sensory evaluation of the wines was performed by using a questionnaire composed of 21 sensory descriptors grouped in two visual descriptors (limpidity, colour intensity), 14 olfactory descriptors: three related to aroma limpidity, aroma intensity and aroma fineness, and 11 aroma terms (citric, green fruit, stone fruit, red fruit, black fruit, tropical fruit, floral, spicy, herbaceous, animal, lactic), three taste descriptors (sweet, acidity, bitter), and two others descriptors (body, flavour quality). Finally, the tasters were instructed to give a global mark of each wine according to their own wine quality perception. All intensity ratings were scored from cero “not perceivable” to ten “very strong”.

A mixture of both intensity and frequency of aroma terms was calculated using the Modified Frequencies formula (MF) proposed by Dravnieks (1985) in order to identified the most important aroma descriptors (Vilanova et al. 2008; Tao et al. 2009). $MF=[F(\%)*I(\%)]^{1/2}$, where F (%) is the detection frequency of an aroma descriptor, and I (%) is the average intensity of that aroma descriptor expresses as percentage of the maximum intensity of the aroma.

Statistical analysis

The modified frequency of the aroma terms and the mean of the rest sensory descriptors obtained in 2006 and 2007 vintages were used to performance the Principal Component Analysis (PCA) for red, white and rose wines. The PCA analysis allowed establishing a relationship between the different sensory descriptors and the studied wines, as well as identifying the most important sensory descriptors in white, rose and red wines. Differences between vintages and between varieties included and excluded from Designation of Origin were checked by ANOVA followed by Tuckey´s HSD test to enable pairwise comparisons of means ($p<0.05$). The modified frequency of the aroma terms and the mean of the rest sensory descriptors, agronomical parameters and chemical analysis were used to analyse the data set using Partial Least Squares regression (PLS), which was applied in order to determinate the relationship between (1) the chemical analysis and sensory data, and (2) agronomical parameters and sensory data. Aroma terms which presented a MF<0.05 were omitted for sensory data statistical analysis. The global mark was used as hedonic measure of the wine quality in order to perform an expert´s preference map, thus it was possible to know which wine was better positioned in relation to the expert preferences and so we could be able to know which wine was better accepted by the wine experts. All statistical analyses were performed using XLSTAT 2009 version.

Results

Sensory descriptions analysis of the tasted wines

Three PCAs were performance following the winemaking protocols (Table 4, Figure 1).

Table 4. Contribution of the variables (%) for the first two axes of the wines characterization following the winemaker protocols. Values > 10% are in bold.

Sensory descriptors	Red wines		White wines		Rose wines	
	F1	F2	F1	F2	F1	F2
Citric	**11.58**	2.72	1.32	**14.45**	9.18	0.13
Green fruit	1.71	2.55	5.75	4.12	8.50	0.08
Stone fruit	2.84	0.11	7.42	0.22	0.09	8.63
Red fruit	0.03	2.29	0.00	0.00	6.20	3.47
Black fruit	5.29	**11.35**	0.00	0.00	5.89	5.36
Tropical fruit	4.43	0.04	6.74	1.82	0.74	**13.01**
Floral	2.42	4.85	7.02	1.17	6.66	0.92
Spicy	0.76	6.21	0.29	**16.87**	8.49	1.41
Herbaceous	9.27	4.67	6.63	2.06	9.34	0.04
Animal	**11.91**	0.37	3.76	8.78	6.68	0.80
Lactic	1.32	**11.26**	5.79	4.04	7.80	0.05
Limpidity	0.10	2.66	1.61	**13.78**	0.83	**12.95**
Colour intensity	2.37	**14.39**	2.50	**11.70**	2.95	0.04
Aroma limpidity	3.14	2.47	7.51	0.01	3.19	3.03
Aroma intensity	1.71	0.04	6.55	2.25	0.01	**12.08**
Fineness	3.87	0.97	4.76	6.43	3.90	8.36
Body	0.31	**19.42**	4.07	8.04	3.89	8.05
Flavour	3.14	**11.32**	7.14	0.88	4.79	5.33
Sweet	**13.19**	0.52	7.50	0.05	0.42	3.53
Acidity	**10.72**	1.52	7.51	0.02	6.86	3.88
Bitter	9.85	0.24	6.12	3.27	3.58	8.84

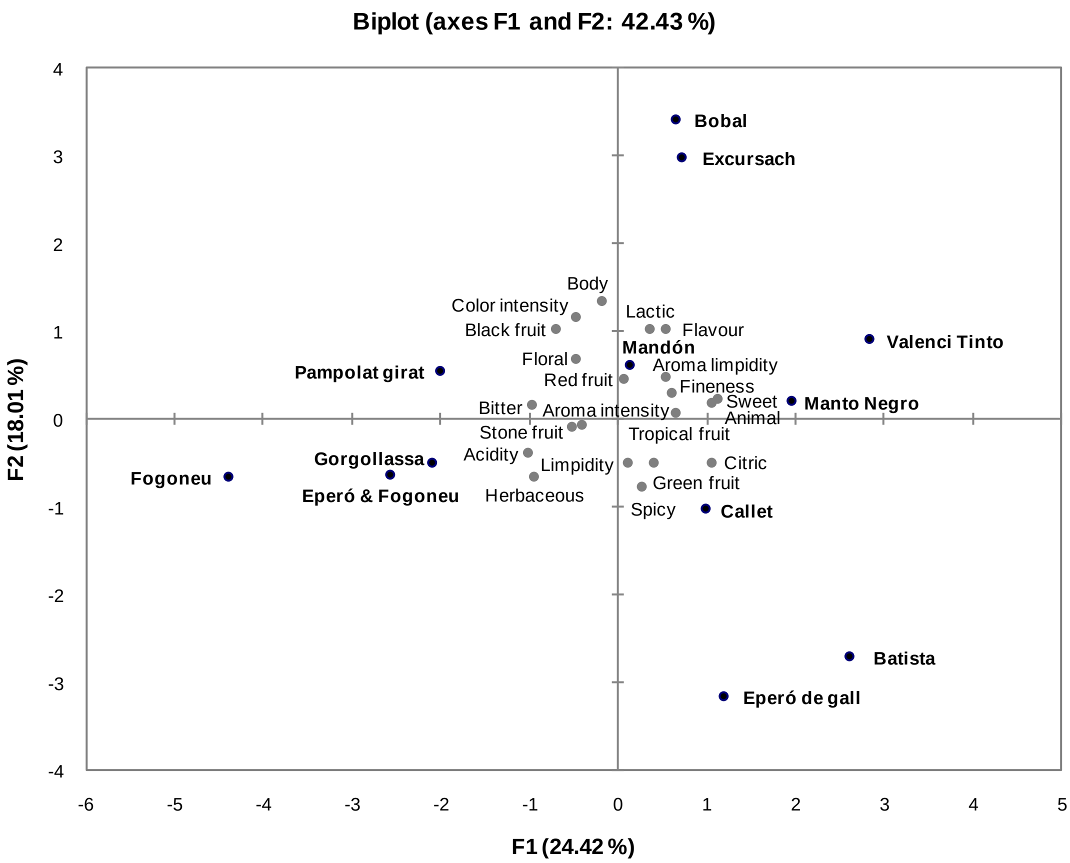

Figure 1. Principal Component Analysis plots for PCA1 and PCA2 of red wines. Symbols: black circles (grapevine varieties) and grey circles (sensory descriptive analysis). The averages of the 2006-2007 vintages descriptors were used for the analysis

In red wines, axis 1 (24% of the variation, Figure 1) was mainly related to sweet descriptors and citric aroma term, producing a separation of Valenci Tinto and Manto Negro on the positive end from Fogoneu and the blend wine made from Eperó de gall and Fogoneu varieties at negative end of PCA1. The axis 2 (18% of the variation) was related to body and colour intensity, separating clearly Bobal and Excursach with grater body of the positive end from the spicy varieties Eperó de gall and Batista at the negative end. Therefore, the first two axes seem to be responding to two relatively independent gradients: the main one (PCA axis 1) associated with sweetened and aroma terms and the second one (PCA axes 2) related to visual and structural descriptors. The red wines, Bobal, Excursach, Mandón, Valenci Tinto and Manto Negro were located in the upper right of the figure and were mainly related to flavour, sweet and aroma limpidity descriptors. Spicy and citric descriptors characterized Callet, Batista and Eperó de gall wines, thus they were placed in the lower right quadrant. Fogoneu, Gorgollassa and the wine made from Eperó and Fogoneu varieties were located in the lower left quadrant, linked mainly to herbaceous and acidity descriptors. Pampolat girat wine was characterized by body, colour intensity, black fruit, floral and bitter descriptors.

The PCA performed for white wines showed that the most discriminatory descriptors for the white wines were aroma limpidity, acidity and sweet descriptors in axis 1 (70% of the variation; Figure 2), and spicy, citric and limpidity in axis 2 (30% of the variation). The first axis seems to be related to taste and aroma terms and the second one with aroma terms. Beba blanca was mapped in the lower right quadrant because of aroma limpidity, colour intensity, sweet, body and floral descriptors, in contrast Quigat, located in the lower left quadrant, was characterized by green fruit and it showed less aroma and flavor. Finally, Pensal Blanca was related to limpidity, spicy, bitter and herbaceous descriptors, thus located in the upper left quadrant.

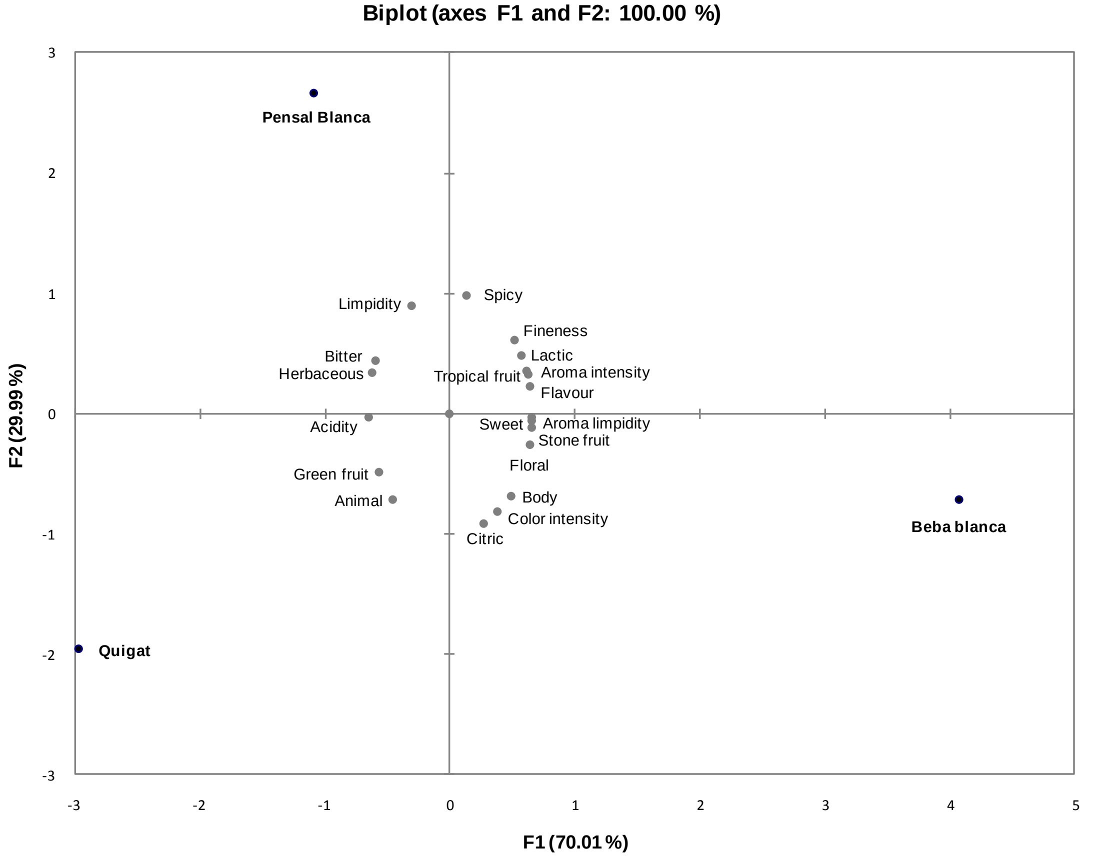

Figure 2. Principal Component Analysis plots for PCA1 and PCA2 of white wines. Symbols: black circles (grapevine varieties) and grey circles (sensory descriptive analysis). The averages of the 2006-2007 vintages descriptors were used for the analysis

Finally, the PCA for rose wines (Figure 3), showed that herbaceous and citric were the most important descriptors for axis 1 (50% of the variation) and tropical fruit, limpidity and aroma intensity for axis 2 (33% of the variation). Beba roja was related to tropical fruit, acidity and citric descriptors. However, Sabaté and Giró were related to limpidity and body, although Giró showed greater black fruit and red fruit descriptors. Finally, Mansés de Tibbus was characterized by aroma intensity, fineness and aroma limpidity.

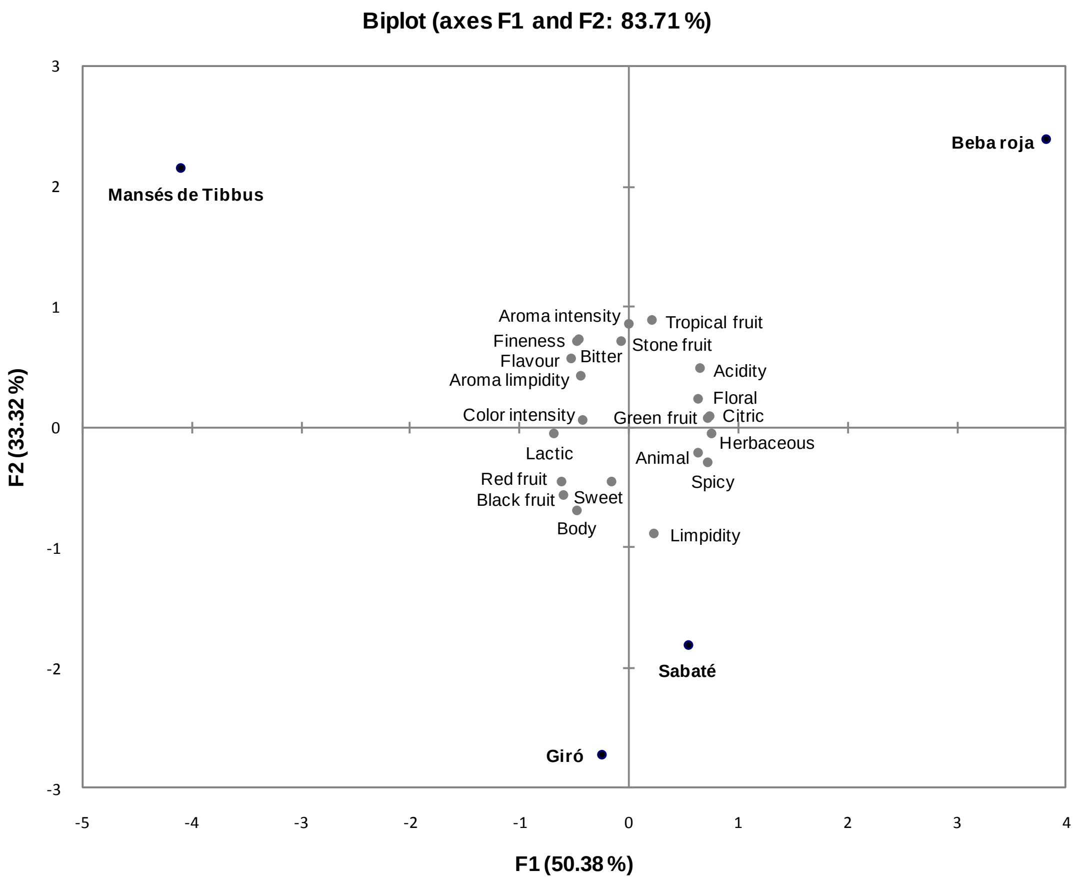

Figure 3. Principal Component Analysis plots for PCA1 and PCA2 of rose wines. Symbols: black circles (grapevine varieties) and grey circles (sensory descriptive analysis). The averages of the 2006-2007 vintages descriptors were used for the analysis

Effect of vintage and Denomination of Origin (DO) over the chemical analysis

According to ANOVA results for the vintage effect over the chemical analysis, in red wines there were significant differences for reducing sugar (r^2=0.36; p<0.05; 1,75 *vs* 1.32 for 2006, 2007 vintage respectively) and total phenolic index (r^2=0.20; p<0.05; 42.83 *vs* 35.91 for 2006, 2007 vintage respectively). On the other hand, the white wines showed significant differences in colour intensity (r^2=0.80; p<0.05; 0.151 *vs* 0.105 for 2006, 2007 vintages respectively) and malic acid (r^2=0.69; p<0.05; 1.13 *vs* 2.33 for 2006, 2007 vintages respectively). However, no significant differences had been found between vintages of the rose wines. Also, there were no significant differences in chemical analyses between varieties included in a Denomination of Origin and those excluded either in red or white wines.

Effect of vintage and Denomination of Origin (DO) over the sensory analysis

According to ANOVA results, the vintage had significant influence over aroma terms in red wines for Fogoneu and Gorgollassa, since significant differences (p<0.05) have been found relating with herbaceous and spicy respectively. Aroma intensity was better scored for Beba roja rose wine in

2007 (r^2=0.30; p<0.05; 4.80 *vs* 6.80 for 2006, 2007 vintages respectively). However no differences were found between the sensorial analyses carried out in 2006 and 2007 vintages in white wines.

There were no differences in the characters related to aroma terms, taste descriptors, body or flavour quality sensory attributes between the varieties included in DO versus those excluded. Although a comparison between red varieties included in a DO and those excluded no significant differences have been found, regarding to white wines, only aroma intensity character was significant different being the wines made from varieties included in DO, Beba and Pensal Blanca were better scored than the excluded ones (r^2=0.11; p<0.05; 6.12 *vs* 4.89, respectively).

Correlation between chemical analysis, agronomical parameters and sensory characterization

In order to evaluate the efficiency of the tasters, PLS were applied to evaluate the correlation between sensory data scored by experts and chemical parameters analysed in the laboratory which also corroborate the classification of PCA. In general, the data obtained by tasters fitted with the chemical analysis carried out in the laboratory (correlation coefficients significantly different from zero, p<0.05, are showed in Tables 5-7).

Table 5. Regression coefficients from the PLS model for the analytical parameters analysed in the laboratory that most contribute in the specific sensory attributes scored by tasters for red wines. Correlation coefficients are in blackest

Sensory attributes	**Total phenolic index**	**Colour intensity**	**Tonality**
Limpidity	-2.753 (-0.60)	-0.650 (-0.42)	
Colour intensity		0.370 (0.97)	
Body		0.699 (0.88)	-0.057 (-0.71)

Table 6. Regression coefficients from the PLS model for the analytical parameters analysed in the laboratory that most contribute in the specific sensory attributes scored by tasters for white wines. Correlation coefficients are in blackest

Sensory attributes	**Alcohol content (%)**	**Relative density**	**Dry extract**	**Volatile acidity**	**Total acidity**	**pH**	**Reducing sugar**	**Malic acid**	**Tonality**
Aroma limpidity	0.162 (0.92)		0.343 (0.96)	0.024 (0.99)	-0.040 (-0.73)	0.024 (0.99)	0.053 (0.96)		0.056 (0.99)
Aroma intensity	0.111 (0.98)		0.235 (0.99)	0.016 (0.97)	-0.028 (-0.94)	0.016 (0.97)	0.036 (0.99)		0.038 (0.92)
Fineness	0.303 (0.96)		0.638 (0.92)	0.044 (0.87)	-0.075 (-0.99)	0.044 (0.86)	0.098 (0.93)		0.104 (0.78)
Body		0.001 (0.95)						0.052 (0.98)	
Flavour	0.200 (0.99)		0.423 (0.99)	0.029 (0.99)	-0.050 (-0.88)	0.029 (0.99)	0.065 (0.99)		0.069 (0.97)
Sweet	0.176 (0.90)	0.001 (0.93)	0.370 (0.95)	0.026 (0.98)	-0.044 (-0.71)	0.026 (0.99)	0.057 (0.95)		0.060 (0.99)
Acidity	-0.157 (-0.94)		-0.332 (-0.98)	-0.023 (-0.99)	0.039 (0.77)	-0.023 (-0.99)	-0.051 (-0.97)		-0.054 (-0.99)
Bitter								-0.152 (-0.99)	

Table 7. Regression coefficients from the PLS model for the analytical parameters analysed in the laboratory that most contribute in the specific sensory attributes scored by tasters for rose wines. Correlation coefficients are in blackest

Sensory attributes	Alcohol content (%)	Volatile acidity	Total acidity	Colour intensity	Malic acid
Colour intensity	0.172 (0.93)			0.026 (0.85)	0.059 (0.84)
Sweet	0.151 (0.83)			0.023 (0.74)	
Acidity		-0.009 (-0.83)	0.036 (0.71)		

It is worth mentioning the correlation between colour intensity analysed in the laboratory and colour intensity scored by tasters for rose and red wines, as well as colour intensity analysed in the laboratory and body scored by tasters. For white and rose wines, it is highlight the positive correlations between total acidity analysed in the laboratory and acidity scored by tasters, reducing sugar analysed in the laboratory and sweet scored by tasters, as well as alcohol content and sweet. Malic acid and relative density were positively correlated with body attribute in white wines. However, volatile acidity was negatively correlated with acidity scored by tasters in white and rose wines as well as pH and acidity attribute for white wine.

With the aim to identify the influence of the agronomical parameters over the sensory wine analysis PLS were performed. Although no correlations were found between agronomical parameters and sensory analysis in red wines, several correlations were found in rose and white wines (correlation coefficients significantly different from zero, $p<0.05$, are showed in Tables 8, 9).

The PLS revealed negative correlations between number of bunches per vine, number of bunches per shoot and green fruit, stone fruit, tropical fruit and floral aroma terms scored by tasters except for green fruit which showed an inverse relationship. However, positive correlations were found between kilograms of pruning wood per kilograms of grape, total woody shoots weight per vine and woody shoots weight and these aroma terms except for green fruit scored by tasters which showed a negative correlation. Although no influence were found between these agronomical parameters and herbaceous term, which was correlated positively with kilograms of grape per vine, bunch weight, number of berries per bunch and negatively correlated with berry weight for white wines, however this attribute was negatively correlated with number of woody shoots per vine in rose wines.

Table 8. Regression coefficients from the PLS model for the agronomical parameters that most contribute in the specific sensory attributes scored by tasters for white wines. Correlation coefficients are in blackest

Sensory attributes	Kg grape/Vine	Number of bunches/Vine	Number of bunches/Shoot	Kg pruning wood/kg grape	Total woody shoots weight/Vine	Woody shoot weight	Bunch weight	Number of berries/Bunch	Berry weight
Citric							-165.645 (-0.90)	-84.563 (-0.86)	
Green fruit		2.074 (0.78)	0.348 (0.78)	-0.181 (-0.81)	-0.116 (-0.99)	-15.335 (-0.95)			
Stone fruit		-1.510 (-0.99)	-0.254 (-0.99)	0.132 (0.99)	0.085 (0.90)	11.167 (0.95)			
Tropical fruit		-1.137 (-0.87)	-0.191 (-0.88)	0.099 (0.90)	0.064 (0.99)	8.405 (0.99)			
Floral		-3.387 (-0.99)	-0.569 (-0.99)	0.295 (0.99)	0.190 (0.82)	25.047 (0.90)			
Herbaceous	0.842 (0.92)						201.992 (0.94)	103.119 (0.97)	-0.288 (-0.93)
Lactic		-2.179 (-0.78)	-0.366 (-0.79)	0.190 (0.82)	0.122 (0.99)	16.111 (0.95)			
Limpidity	0.089 (0.94)						21.366 (0.92)	10.908 (0.88)	-0.030 (-0.94)
Colour intensity	-0.044 (-0.98)						-10.609 (-0.96)	-5.416 (-0.94)	0.015 (0.98)
Aroma limpidity		-0.273 (-0.98)	-0.046 (-0.99)	0.024 (0.99)	0.015 (0.93)	2.019 (0.98)			
Aroma intensity		-0.165 (-0.86)	-0.028 (-0.86)	0.014 (0.89)	0.009 (0.99)	1.222 (0.98)			
Fineness		-0.397 (-0.68)	-0.067 (-0.69)	0.035 (0.72)	0.022 (0.95)	2.932 (0.90)			
Body	-0.078 (-0.99)						-18.623 (-0.99)	-9.507 (-0.99)	0.027 (0.99)
Flavour		-0.312 (-0.92)	-0.052 (-0.92)	0.027 (0.943)	0.017 (0.99)	2.306 (0.99)			
Sweet		-0.297 (-0.99)	-0.050 (-0.99)	0.026 (0.99)	0.017 (0.92)	2.199 (0.97)			
Acidity		0.260 (0.98)	0.044 (0.98)	-0.023 (-0.99)	-0.015 (-0.95)	-1.921 (-0.99)			
Bitter	0.204 (0.96)						48.875 (0.97)	24.951 (0.99)	-0.070 (-0.96)

Table 9. Regression coefficients from the PLS model for the agronomical parameters that most contribute in the specific sensory attributes scored by tasters for rose wines. Correlation coefficients are in blackest

Sensory attributes	Kg grape/Vine	Number of bunches/Vine	Number of bunches/Shoot	Kg pruning wood/kg grape	Total woody shoots weight/Vine	Number of woody shoot/Vine	Bunch weight	Number of berries/Bunch	Berry weight	Must yield
Citric						-0.455 (-0.88)				
Red fruit							41.988 (0.65)	76.381 (0.71)		
Black fruit						0.916 (0.99)				
Tropical fruit		-2.460 (-0.40)	-0.146 (-0.99)							
Floral						-1.909 (-0.85)				
Herbaceous						-1.346 (-0.81)				
Limpidity		0.324 (0.60)	0.023 (0.82)							
Aroma limpidity										0.877 (0.92)
Aroma intensity		-0.311 (-0.75)								
Fineness			-0.016 (-0.52)	0.033 (0.54)						
Body		0.397 (0.98)	0.021 (0.92)			0.191 (0.94)				
Flavour					0.064 (0.98)					0.812 (0.79)
Sweet	0.077 (0.77)							11.129 (0.99)	-0.083 (-0.91)	
Acidity						-0.120 (-0.99)				
Bitter			-0.028 (-0.55)	0.057 (0.57)						

Number of bunches per vine was negatively correlated with aroma intensity in white and rose wines. In white wines, number of bunches per vine and number of bunches per shoot were also negative correlated with sweet. Body attribute scored by tasters was negatively correlated with kilograms of grape per vine for white wines, however in rose wines this attribute was positively correlated with number of bunches per vine and number of bunches per shoot.

Analysis of the preference map

Tasters quantified each wine given a global mark which was used as hedonic measure of the wine quality. Therefore, it was possible to map the wines according to expert´s preferences. As a consequence, two preferences maps were performed one for red wines and other one for white and rose wines. In red wines, four clusters were performed (Table 10, Figure 4).

Table 10. Wines sorted by increasing expert´s preference order in red wines

Cluster1	Cluster2	Cluster3	Cluster4
Eperó de gall	Fogoneu	Fogoneu	Eperó de gall
Batista	Eperó&Fogoneu	Eperó&Fogoneu	Batista
Callet	Gorgollassa	Gorgollassa	Callet
Eperó&Fogoneu	Pampolat girat	Pampolat girat	Eperó&Fogoneu
Manto Negro	Eperó de gall	Mandón	Gorgollassa
Gorgollassa	Callet	Bobal	Manto Negro
Fogoneu	Batista	Excursach	Fogoneu
Valenci Tinto	Mandón	Callet	Valenci Tinto
Mandón	Manto Negro	Eperó de gall	Mandón
Pampolat girat	Excursach	Manto Negro	Pampolat girat
Excursach	Bobal	Batista	Excursach
Bobal	Valenci Tinto	Valenci Tinto	Bobal

The most preference wines were Bobal and Excursach for cluster 1 and 4, however Valenci Tinto and Bobal were better scored by Cluster 2 and Valenci Tinto and Batista by cluster 3. Therefore the wines Bobal (allowed in six Spanish DOs), Excursach, Valenci Tinto and Mandón were located in the better profile of the expert's preference (80-100% preference). In contrast two wines made from varieties allowed in Majorcan DOs, Manto Negro and Callet, were mapped in the 40-60% of the experts preferences, as well as Pampolat girat wine made from a not allowed variety in Spanish DOs. It is worth comment that Fogoneu wine was showed the lowest rating among the variety included in Majorcan DOs. It was a bit better located in the preference map than Gorgollassa and Eperó de gall. However, the wine made with the blend of Eperó de Gall and Fogoneu varieties was rejected by experts, since it was the less preferred, 0-20%.

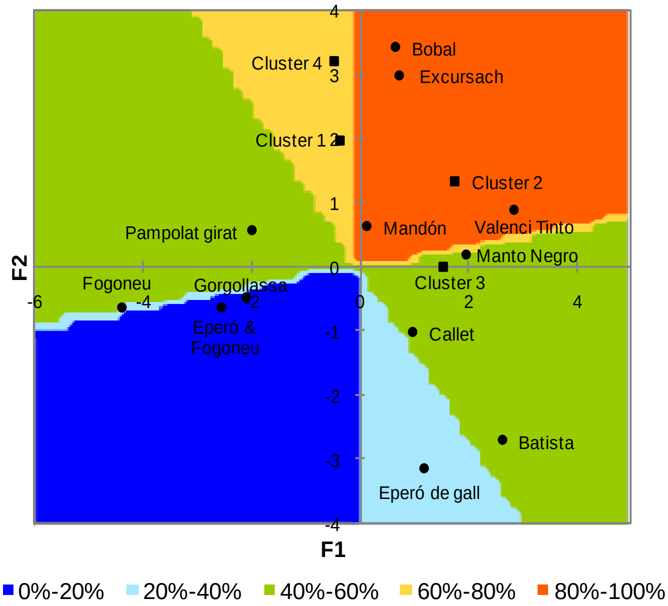

Figure 4. Preference mapping and contour plot for red wines projected on PCA axes. Symbols: black circles (grapevine varieties) and black squares (different expert´s clusters). The averages of the 2006-2007 vintages descriptors were used for the analysis

According with expert´s preference map performed from white and rose wines, three clusters were obtained, small different between them were detected. The first and second cluster preferred Beba blanca and Mansés de Tibbus wines, however the third cluster preferred the wines made from Mansés de Tibbus and Giró (Table 11, Figure 5). Therefore Mansés de Tibbus rose wine was the only one located in the better position of the expert´s preferences (80-100% preference). Beba blanca wine, variety allowed in one Spanish DO, located in the lower right quadrant, meaning that 60-80% of the experts accepted this wine. Giró and Sabaté rose wines were mapped in the 20-40% of the experts preference map. Pensal Blanca, variety allowed in Majorcan DOs, was accepted by around the 40% of the expert, since this wine was located in the border between 20-40% and 40-60% of the contour plot. Giró and Sabaté wines were preferred by 20-40% of the experts. Nevertheless, Beba roja and Quigat were the wines rejected by experts.

Table 11. Wines sorted by increasing expert´s preference order in white and rose wines

Cluster1	Cluster2	Cluster3
Giró	Beba roja	Pensal Blanca
Beba roja	Quigat	Quigat
Sabaté	Giró tinto	Beba blanca
Quigat	Sabaté	Beba roja
Pensal Blanca	Pensal Blanca	Sabaté
Mansés de Tibbus	Mansés de Tibbus	Giró
Beba blanca	Beba blanca	Mansés de Tibbus

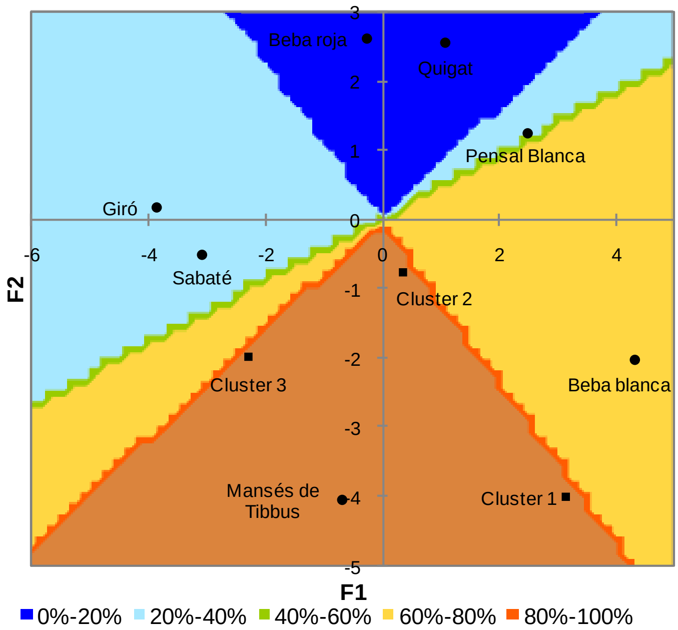

Figure 5. Preference mapping and contour plot for white and rose wines projected on PCA axes. Symbols: black circles (grapevine varieties) and black squares (different expert´s clusters). The averages of the 2006-2007 vintages descriptors were used for the analysis

Discussion

Sensory descriptions analysis of the tasted wines

Our results reveal that tasters differentiated the wines according to sensory attributes as it has been appointed in other studies (Lund et al. 2009; Muñoz-González et al. 2011). However, according to PCA result, olfactory, taste and visual characters showed different weight for red, white and red wines. Taste attributes were more significant in red wines, mainly because these attributes are easier to asses than the aroma ones (Koussissi et al. 2003). In contrast, the attributes related to aroma terms, mainly citric, spicy and herbaceous ones, were more significant for white and rose wines as it has been appointed by other authors for different wines (Koussissi et al. 2003; Nurgel et al. 2004; Scacco et al. 2007). Thus, as a view of these results, the questionnaire could be quantified efficient for the tasted wines.

Effect of vintage and Denomination of Origin (DO) over the chemical and sensory analysis

Good reproducibility of chemical and sensory analysis results was obtained for the two vintages, despite of the vintage influenced over both analyses. In this study, significant differences have been found in analytical data between the two studied vintages for reducing sugar, total phenolic index in red wines, and colour intensity and malic acid in white wines out 12 chemical data analyzed. However, it seems that these differences have not been high enough to be scored by experts in the sensorial analysis. On the other hand, differences between wines sensorial analysis between the two vintages have been also established in this work as well it has been appointed by other authors (Fischer et al. 1999; Vilanova et al. 2008). These aroma attributes were related to aroma intensity in white wines and two aroma terms scored by tasters for Fogoneu and Gorgollassa red wines.

Relating to the influence of the DO over wines analyses, only aroma intensity out of 21 sensory attributes analyzed showed significant different between white wines made from varieties included in DO for those excluded. These results are in agree with other authors who have appointed that differences between wines including or not in a Quality Demarcation are not easy to assess (Maitre et al. 2010). Therefore, the effect of vintage is more important than the Denomination of Origin over both chemical and sensory characterization for this group of wines.

Correlation between chemical analysis, agronomical parameters and sensory characterization

Despite the tasters were not training, there was a correlation between wine parameters analyzed in the laboratory and those scored by the tasters as sweet, acidity or colour intensity, fitted thoroughly overcoat in white and rose wines. These results emphasize the good outcome obtained by expert´s tasters (Perrin et al. 2007). However, the correlations in the attributes related to taste were not stronger in red wines; as a consequence of the interactions between phenolic compounds and mouth-fell properties (Vidal et al. 2004; Preys et al. 2006). In contrast, the correlations showing a value over 71% between taste parameters and those obtained by instrumental analysis as colour intensity-colour intensity, total acidity-acidity, pH-acidity, reducing sugar-sweetness, were in line with other studies (Boselli et al. 2004; Blackman et al. 2010; Sáenz-Navajas et al. 2010). However, the correlation between alcohol content and sweetness have not been appointed for Blackman et al. (2010) or Sáenz-Navajas et al. (2010), conversely to Zamora et al. (2006), these discrepancies could be due to the different alcohol content and reducing sugar among the studied wines. The relationship between colour intensity and body is linked to the phenolic content since these compounds have a direct effect on the sensation of weigh or body (Jackson 2008).

There were no correlations between agronomical characterization and sensorial analysis in red varieties, suggesting that it might be caused by the high variability of the studied varieties. However, in white and rose varieties we found a strong relationship between agronomical characterization and sensory attributes. The studies relating agronomical data with wine sensory characterization are not so numerous, although field parameters as yield (Chapman et al. 2004), vine density (Koussissi et al. 2008), vineyard mechanization (Diago et al. 2010) or water status (Hakimi Rezaei and Reynolds 2010) have been pointed out to be related to wine quality. Sensorial analysis of wines respond to yield management (Chapman et al. 2004), since herbaceous aroma term and flavour were related to kg of grape per vine as well as flavour was linked to number of bunches per vine and number of bunches per shoot, these results are in concordance with Chapman et al. (2004) who found similar results for Cabernet-Sauvignon wines in Napa Valley (California, EEUU). In this study, it is proved that the vegetative (hence “total woody shoots weight per vine” and “woody shoot weight”) and productive parameters (hence “number of bunches per vine” and “number of bunches per shoot”) influence inversely over aroma and taste scored by experts, and also their balance (hence “Kg pruning wood per kg grape”) are linked to the wines sensory attributes. All of these parameters are linked directly with must characteristics, as it is to be expected to final wine (Jackson 2008).

Analysis of the preference map

The utility of the preference map has been probed in wine to map the wines according to tasters scores (Lund et al. 2009). In this study, it was possible to map the wines according to expert´s preferences. Therefore, the varieties located in the best position of the expert preference map should be considered by technicians due to their quality, sometimes even better mapped than the wines done from varieties allowed in Spanish DOs. The wines made from varieties not included in DOs had sensorial and chemical results close to the wines made from varieties included in Spanish DOs.

Conclusions

Both chemical and sensory characterizations were influenced by vintage and Denomination of Origin effects, however vintage effect was more significant over this group of wines. Therefore important differences have not been found between sensory study of wines made from minor varieties not included in Denomination of Origin and those excluded.

This study corroborates the idea that it necessary to achieve a balance between vegetative and productive growth, since not only the productive, also the vegetative parameters, sways over the final wine sensorial characterization, which indicates that the management of these parameter might modify the original sensory profile. Further studies are needed to improve the knowledge of the way of how agronomical parameters influence over the final wine quality.

In summary, in this work, which is the first sensorial analysis of these minor varieties, highlights the good acceptation of these wines by wine experts, since the tasters scored the wines made from minor varieties in similar way as the varieties included in Denomination of Origin, as the cases of Excursach, Mandón or Pampolat girat for red wine. Therefore, the possibilities of the minor varieties for the wine market have been shown. This information would gain a more interest of minor or autochthonous wine varieties being key for their preservation.

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Chapter 8

Trapped

Synthesis

Synthesis

Based on historical references, one of the most interesting results was the high grapevine diversity found in the Balearic Islands despite of its small geographic area. The islands are an important stronghold for several cultivars conserving several unique genotypes (Chapter 2 and Chapter 4). However, the loss of grapevine diversity has been quantified for the first time, highlighting an important loss around 50% of grapevine diversity in around 300 years. This problem is also common in other viticulture areas (Sladonja et al. 2007; Carimi et al. 2010). Unfortunately, nowadays some of the antique cultivars are under risk of extinction, as a consequence it is recommended almost the maintenance of the cultivars in germplasm repositories before the loss of the antique cultivars is irreversible.

The main causes of grapevine diversity loss appointed in the Balearic Islands, as powdery mildew (*Erysiphe necator* Schwein) or the appearance of the phylloxera plague, have been also highlighted by others authors (Martínez and Pérez 2000; This et al. 2006). In any case, the greatest change over the Balearic viticulture was dated before the phylloxera plague arrived to the islands contrary to what was thought. The great Balearic viticulture change was related to the phylloxera crisis in France; during this period some varieties as Callet and Manto Negro were favoured due to their high production (Chapter 2), these cultivars have been conserved until nowadays. Also just before and just after the phylloxera plague arrived to the islands, there were a high number of accessions which could not been identified. These results confirm the theory about the lack of ampelography knowledge (Aradhya et al. 2003), partially due to the foreign varieties introduced from the Iberian Peninsula (as Garnacha, synonymy Grenache; Carretero et al. 1875) or from France (as Cinsaut; Satorras 1892) during a relative short time period. Before the phylloxera plague arrived to the islands, the cultivars were introduced mainly from France (Ballester 1911) to satisfy the new demands of the wine consumers, whereas some cultivars were introduced to reconstruct the disappeared vineyard area after the arrival of the phylloxera plague. For these reasons, numerous synonyms and homonyms have been found (Chapter 2, Chapter 3, Chapter 4), some of them caused by orthographic or phonetic name variations (Chapter 2) as other authors has been appointed (Cipriani et al. 2010). Antique references have been found for all the studied accessions and all of them sited the varieties in the Balearic Islands, so it seems to be proved the relationship between the studied cultivars and the Balearic Islands (Chapter 2, Chapter 4).

The combination of the ampelographic and genetic characterization has allowed the identification of the studied accessions (Chaper 3, Chapter 4). As well, the ampelographic descriptions have been key to identify the references found in the antique bibliography (Chaper 2), since more than 75% of the found cultivars have been identified using this technique.

The use of ampelographic descriptions was also the key for the differentiation between Beba blanca and Beba roja based on berry colour (white and rose respectively). Beba blanca could be considered a somatic mutant from Beba roja, therefore these two cultivar should be considered as different cultivars (Laiadi et al. 2009). The ampelographic descriptions have been appointed to be not objective (Dettweiler 1993), however the results obtained in this research point in opposite direction, due to the high reproducibility level of the ampelographic results (Chapter 3), and it has been also demonstrated that only three characters, colour of the young leaf's upper side (OIV-051), juiciness of the flesh (OIV-232) and firmness of the flesh (OIV-235) out of the 46 qualitative parameters studied were not easy to asses by the ampelographers. However, the increased of the ampelographer's experience, as it has been appointed by Ortiz et al. (2004), and the correct selection of the plant material are factors that improved the correct ampelographic descriptions.

The genetic characterization, using microsatellite markers (SSR), establishes a strong genetic relationship between all the studied cultivars, possible due to the isolation of the geographic area, as it has been observed in other islands species (Sales et al. 2001). This genotypical fact could be also related to the high phenotipical similarity showed by the studied cultivars (Chapter 3, Appendix II).

Despite it is difficult to establish precisely the origin of the grapevine cultivars due to the high material exchange (This et al. 2006), it seems that the dispersal of the studied varieties are related with historical human movements and migrations (Costantini et al. 2005). The material exchange in the Balearic Islands occurred in three periods, the first one around VII century related to Islam expansion, the second one around XIII-XV centuries related to the expansion of the Kingdom of Aragón (current Spain) over the Mediterranean Basin, and the third one in the XIX century related to phylloxera crisis (Chapter 2, Chapter 4). The historical references and the use of the microsatellite analysis of some of the studied varieties has allowed to prove the origin of some cultivars, as for Callet or Manto Negro, however the origin of other varieties has been discussed, as Beba or Excursach, and unfortunately, the origin of certain varieties remaining unknown (Chapter 2, Chapter 4). The use of microsatellite analysis has also confirmed the existence of two gene pools, one of them related to Callet cas Concos, an unique genotype collected in a prospection in the Balearic Islands, standing out the high value of the unknown cultivars.

In order to know the possibilities of the minor varieties, the agronomic behaviour has been studied. The cultivars have shown a wide range of possibilities in their both agronomic and oenological characterization. Vinegrowers and winemakers can select grapevine cultivars more or less productive, with several must characterizations or with different grape potential aroma to produce wines with diverse final characteristics (Chaper 6, Chapter 7, Appendix III). Several varieties, which are not allowed in any Demarcation of Quality, have shown high resistance to powdery mildew (*Erysiphe necator* Schwein.) on both bunches and leaves (Chapter 5). This character seems to be important for the conservation of these cultivars, since the minor varieties conserved in the Balearic Islands have shown high resistance to powdery mildew. Therefore it is possible to select cultivars with different susceptibility. The selection of more resistant cultivars to powdery mildew implies the reduction of control treatments against this disease.

The typicality of wines is influenced by a large number of factors, as grapevine (Esti and Tamborra 2006), environmental conditions (Ribéreau-Gayon et al. 2006), agronomical management (Chapman et al. 2004) or vintage (Maitre et al. 2010). All of these factors are also related to final wine quality and have been analyzed in this study.

Grapevine cultivar influenced the typicality of wines based mainly on two aspects: the grape colour of the cultivar, and grape aroma characterization (Chapter 6). The relationship between grape variety and wine obtained has been previously proved (Ugliano and Moio 2008). However in this study the wine made from white and red varieties can be clearly differentiated based on different aroma composition of grape cultivars (Chapter 7).

The differences between red and white grapevines are mainly caused by a mutation on two genes (Walker et al. 2007), and it seems that this mutation play also an important role in the pathways to synthesize several aromatic compounds, as benzenic which seems to be lead with the synthesis of polyphenols (Haslam 1998; Dudareva et al. 2006), or terpenic compounds which has been appointed to have a genetic component (Chen et al. 2011; Chapter 6). These compounds have been key between black and white grape cultivars differentiation. When several accessions of Beba and Giró varieties were studied, differences among their aromatic profiles were found. Therefore, it seems possible to select clones with higher aroma potential to obtain wines with a different aroma profile (Botelho et al. 2010). The terpene compounds were also key to the differentiation of the origin of several accession of Beba based on aroma characterization. These differences between grapevine clones based on aroma compounds has been also appointed by other authors (Botelho et al. 2010), as well as the influences of the geographic origin over the aroma compounds in must (Mulet et al. 1992; Zamuz and Vilanova 2006). Therefore, the genetic differentiation between red and white cultivar might be also related to the different grape aroma compound found in white and red varieties.

Related to the environmental conditions, the effect of hailstorm phenomenon was also studied, underlining its influenced over the agronomic characterization. Hailstorm reduced Kilograms of grape per vine, number of bunches per shoot and must pH, and increased woody shoot weight and total acidity (Chapter 3). Some wine sensorial attributes seem to be also related to agronomic parameters, such as tropical fruit aroma term or aroma intensity, which were influenced by Kilograms of grape per vine, number of bunches per shoot and total woody shoots weight per vine (Chapter 7). It is proved that the vegetative parameters, such as total woody shoots weight per vine and woody shoot weight, and productive parameters such as number of bunches per vine and number of bunches per shoot, influenced inversely over aroma and taste scored by wine experts, pointing out that balance between vegetative and productive growth might exist. Therefore the management of these agronomic parameters might modify the original sensory profile (Chapter 7). These results suggest that environmental conditions and agronomic management are related with final wine quality.

Despite of the vintage influenced over both chemical and sensory analysis, good reproducibility of the results was obtained for the two vintages analyzed in this study. The vintage influenced over both wine chemical and sensory analysis. Similar results have been also appointed by other authors (Fischer et al. 1999). In this study, wine chemical analysis, such as reducing sugar or total phenolic index in red wines, and colour intensity and malic acid in white wines, were influenced by the vintage. However, it seems that these differences have not been high enough to be scored by experts in the sensorial analysis. On the other hand, related to sensory analysis, differences between sensorial wines analysis between two vintages have been also established, such as aroma intensity in white wines and two aroma terms scored by tasters for Fogoneu and Gorgollassa red wines (relating with herbaceous and spicy respectively; Chapter 7).

Beside the quality of wine is difficult to assay (Charters and Pettigrew 2007), quantitative and descriptive analysis are the most used methods in wine sensory characterization (Murray et al. 2001). In this study, wine sensory characterization was carried out over wines made from 18 minor varieties. Consequently, based on expert wine scores, it was possible to map the wines according to their preferences. Therefore, the varieties located in the best position of the expert preference map should be considered by technicians due to their quality.

Other parameter studied was the effect of the inclusion or not of a variety in a Denomination of Origin (Chapter 6, Chaper 7). Related to the grape aroma profile, some minor varieties excluded from DO (i.e. Argamusa, Gorgollassa and Pampolat girat) appeared to be more aromatic than the varieties included in DO. However, the wines made from varieties not included in DOs had sensorial and chemical results close to the wines made from varieties included in Spanish DOs, since no differences were found related to wine chemical analysis. Also only aroma intensity out of 21 sensory attributes analyzed showed significant differences between white wines made from varieties included in DO than those excluded. Taking into account the scores given by the wine experts in sensory characterization, the wines made from minor varieties were sometimes even better mapped (as Excursach of Mandón) than the wines done from varieties allowed in Spanish DOs (as Fogoneu). These results are in agree with other authors who have appointed that differences between wines included or not in a Quality Demarcation are not easy to assess (Maitre et al. 2010). As a consequence, minor varieties could be taken into consideration for the development of new wine market strategies, which could also play an important role in the conservation of these cultivars.

Recommendations for practices

Historical references and microsatellite analysis clarify the relationship between cultivar and site, which is the link between “terroir” and historical background. Agronomic characterization and resistance to powdery mildew give an idea about the agronomic behaviour. Aromatic grape and chemical wine characterization, as well as sensory analysis of the made wines with wine expert preferences conditioned the oenological potential of the studied cultivars. On the basis of these results seven cultivars could be recommended to improve the wine quality and conservation of the viticulture heritage of the Balearic Islands. These cultivars, in order of preference are Argamusa and Quigat for white vinifications, Gorgollassa, Pampolat girat, Mandón and Excursach for red vinifications and Mansés de Tibbus for rose vinifications.

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Chapter 9

At the end of the road

Conclusions

Conclusions:

1.- High grapevine diversity (*Vitis vinifera* L.) has been found in the Balearic Islands, despite of its small geographic area. The material exchange in the Balearic Islands occurred in three periods, the first one around VII century related to Islam expansion, the second one around XIII-XV centuries related to the expansion of the Kingdom of Aragón (current Spain) over the Occidental Mediterranean Basin, and the third one in the XIX century related to phylloxera crisis.

2.- The loss of grapevine diversity is evident; half of the identified cultivars growing in natural conditions have been lost in approximately 300 years. The high rate of grapevine loss needs to provide roles of conservation. Special attention is recommended for the unique genotypes not found around the world out of the Balearic Islands. They must to be preserved in grapevine resources before the loss of these cultivars is irreversible.

3.- A high reproducibility level of the ampelographic results has been found. Only three characters: colour of the young leaf's upper side (OIV-051), juiciness of the flesh (OIV-232) and firmness of the flesh (OIV-235) out of the 46 qualitative parameters studied were not easy to asses by the ampelographers. The increased of the ampelographer's experience and the adequate selection of the plant material are factors that improve the correct ampelographic description.

4.- Three new synonyms around the Mediterranean Basin have been found: Excursach (Balearic Islands, Spain) matched with Murescu (Corsica, France); Pampolat girat (Tarragona, Spain) with Cruixent (Corsica, France), and Giró Ros (Balearic Islands; Spain) with Giro sardo (Sardinia, Italy).

5.- Two lineages (kin-groups) were pointed out, the first one related with Callet Can Concos variety and the second one with Hebén variety. Thirteen putative parentages have been obtained; six of them showed full compatibility for all nuclear microsatellites studied and for the chlorotype of both parents (Callet, Eperó de Gall, Gafarró, Manto Negro, Unknown 1 and Viñaté).

6.- The combination of ampelography, microsatellite analysis and synthesis of historical references of the cultivars has shown to be excellent tools for a good identification of grapevine material and to establish a correct regional viticulture.

7.- Thirty three out 159 varieties studied have shown high resistance to powdery mildew (*Erysiphe necator*) in both bunch and leaf, being 10 cultivars from the Balearic Islands. Beba blanca was the only variety from the Balearic Islands which showed high susceptibility in both bunch and leaf to this disease.

8.- Cultivar, berry colour, and cultivar adaptations to location and environmental conditions play an important role in the metabolic pathways to obtain the aromatic compounds. These metabolic pathways seem to have a genetic component.

9.- Based on the results obtained in this study related with the relationship between cultivar and site (hence historical references and microsatellite analysis), agronomic behaviour (hence agronomic characterization and resistance to powdery mildew), and oenological potential of the studies cultivars (hence aromatic grape and chemical wine characterization, sensory analysis of the made wines and wine expert preferences) seven cultivars could be recommended for the improvement of wine quality and conservation of the viticulture heritage of the Balearic Islands. These cultivars, in order of preference are: Argamusa and Quigat for white vinifications, Gorgollassa, Pampolat girat, Mandón and Excursach for red vinifications and Mansés de Tibbus for rose vinifications.

Conclusiones:

1.- Se ha encontrado una elevada diversidad genética de variedades de vid (*Vitis vinifera* L.) en las Islas Baleares a pesar de su reducida área geográfica. El intercambio de material vegetal en las Islas Baleares se ha producido en tres periodos, el primero alrededor del siglo VII relacionado con la expansión del Islam, el segundo entre los siglos XIII-XV, relacionado con la expansión del Reino de Aragón en el Mediterráneo occidental, y el tercero sobre el siglo XIX relacionado con la crisis filoxérica.

2.- La pérdida de diversidad de variedades es evidente, en aproximadamente 300 años se ha perdido en condiciones naturales la mitad de las variedades de vid que se cultivaban históricamente. Esta elevada pérdida de variedades obliga a la recomendación de políticas de conservación. Los genotipos únicos que no se encuentran en otras partes del mundo fuera de las Islas Baleares deben ser conservados en bancos de germoplasma antes de que la pérdida de estas variedades sea irreversible.

3.- Los resultados de descripciones ampelográficas han mostrado un elevado nivel de reproducibilidad. Sólo tres caracteres, correspondientes a color del haz de la hoja joven (OIV-051), suculencia de la pulpa (OIV-232) y firmeza de la pulpa (OIV-235), de los 46 parámetros cualitativos estudiados no fueron fáciles de evaluar por los ampelógrafos. La experiencia de los ampelógrafos y la correcta selección del material son factores que mejoran la descripción ampelográfica.

4.- Se han encontrado tres nuevas sinonimias alrededor de la cuenca mediterránea: Excursach (Islas Baleares, España) coincide con Murescu (Córcega, Francia); Pampolat girat (Tarragona, España) con Cruixent (Córcega, Francia), y Giró Ros (Islas Baleares, España) con Giro sardo (Cerdeña, Italia).

5.- Se han encontrado dos linajes (“kin-groups”), el primero relacionado con la variedad Callet Can Concos y el segundo con la variedad Hebén. Se han obtenido también 13 posibles parentescos, seis de ellos han mostrado compatibilidad completa para ambos parentales en todos los microsatélites nucleares y clorotípicos estudiados (Callet, Eperó de Gall, Gafarró, Manto Negro, Desconocida 1 and Viñaté).

6.- La combinación de las descripciones ampelográficas, los análisis genéticos de marcadores moleculares y las referencias históricas han mostrado ser unas herramientas excelentes para realizar una buena identificación de las variedades y para establecer una correcta viticultura regional.

7.- Treinta y tres de las 159 variedades estudiadas han mostrado una alta resistencia al oídio (*Erysiphe necator*) tanto en racimo como en hoja, siendo 10 de estas variedades procedentes de las Islas Baleares. De las variedades estudiadas procedentes de las Islas Baleares, solamente la variedad Beba blanca mostró alta susceptibilidad tanto en racimo como en hoja a esta enfermedad.

8.- La variedad, color de la baya y adaptaciones locales de la variedad a condiciones ambientales juegan un importante papel en la rutas metabólicas para obtener compuestos aromáticos. Estas rutas metabólicas parecen tener un componente genético.

9.- Teniendo en cuenta los resultados obtenidos en este estudio respecto a la relación entre cultivar y territorio (referencias históricas y análisis microsatélites), comportamiento agronómico (caracterización agronómica y resistencia al oídio), y potencial enológico de las variedades estudiadas (caracterización aromática de uvas, caracterización química y sensorial de los vinos realizados y preferencia de los catadores), se podrían recomendar siete variedades para mejorar la calidad de los vinos y la conservación del patrimonio vitícola de las Islas Baleares. Estas variedades, en orden de preferencia, son: para vinificaciones en blanco Argamusa y Quigat, para vinificaciones en tinto Gorgollassa, Pampolat girat, Mandón y Excursach, y para vinificaciones en rosado Mansés de Tibbus.

Appendix I: Plant material

Table 1. Plant material analyzed in this study from the Balearic Islands (except * from Levante area, ** from Girona, *** from Tarragona) located at the *Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain). Local names=names of the accession; Acc.: accession. Variables: Historical references; Ampelographic description; Genetic analysis; Resistance to powdery mildew (*Erysiphe necator* Schwein.; Resist. powd. mildew); Agronomic characterization (Agron. charact.); Must variables (Must var.); Grape aroma characterization (Grape aroma charac.) and Sensorial wine analysis (Sensor. wine analysis). Accession analyzed (✓)

Local name	Acc.	Berry color	Prime name at VGB "Finca El Encín"	Historical Reference	Ampelographic description[1]	Genetic analysis
Argamusa	E34	white	Argamusa	✓	no	✓
Calop	E32	white	Beba	✓	✓	✓
Calop blanco	E43	white	Beba	✓	✓	✓
Corazón de Ángel *	O16	white	Beba	✓	✓	✓
Grumiere blanco *	O44	white	Beba	✓	✓	✓
Jaumes	E14	white	Beba	✓	✓	✓
Massacamps	E38	white	Quigat	✓	✓	✓
Mateu **	E39	white	Beba	✓	✓	✓
Pensal blanco, Moll, Prensal blanco	SCL	white	Pensal Blanca	✓	✓	✓
Quigat	E33	white	Quigat	✓	✓	✓
Valenci blanco *	O17	white	Beba	✓	✓	✓
Viñaté	E45	white	Viñaté	✓	no	✓
Calop rojo, Calop roig	E19	rose	Beba roja	✓	✓	✓
Batista	E23	black	Batista	✓	✓	✓
Beba negra, Calop negro, Calop negre	E18	black	Valenci Tinto	✓	✓	✓
Boal	E21	black	Bobal	✓	✓	✓
Cabellis	E20	black	Manto Negro	✓	✓	✓
Callet	E26	black	Callet	✓	✓	✓
Eperó de Gall, Esperó de gall	E37	black	Eperó de gall	✓	✓	✓
Excursach, Escursach	E27	black	Excursach	✓	✓	✓
Fernandella	E22	black	Fernandella	✓	no	✓
Fogoneu	E24	black	Fogoneu	✓	✓	✓
Fogoneu francés	E16	black	Fogoneu	✓	no	✓
Galmete	E45	black	Mandón	✓	no	✓
Giró	E36	black	Mancés de Capdell	✓	✓	✓
Gorgollasa, Gorgollassa	E25	black	Gorgollassa	✓	✓	✓
Mancés de Capdell	E31	black	Mancés de Capdell	✓	✓	✓
Mansés de tibbus, Mancés de Tibbus	E30	black	Mansés de Tibbus	✓	✓	✓
Manto negro	E29	black	Manto Negro	✓	✓	✓
Pampolat Girat***	H24	black	Pampolat girat	✓	✓	✓
Sabaté, Sabater	E35	black	Sabaté	✓	✓	✓
Vinaté, Vinater	E11	black	Bobal	✓	✓	✓

Local name	Acc.	Berry color	Prime name at VGB “Finca El Encín”	Resist. powd. mildew[2]	Agron. charact.[3]	Must var.	Grape aroma charac.	Sensor. wine analysis[4]
Argamusa	E34	white	Argamusa	✓	no	✓	✓	no
Calop	E32	white	Beba	✓	✓	✓	✓	✓
Calop blanco	E43	white	Beba	✓	✓	✓	✓	✓
Corazón de Ángel *	O16	white	Beba	✓	✓	✓	✓	✓
Grumiere blanco *	O44	white	Beba	✓	✓	✓	✓	✓
Jaumes	E14	white	Beba	✓	✓	✓	✓	✓
Massacamps	E38	white	Quigat	✓	✓	✓	✓	✓
Mateu **	E39	white	Beba	✓	✓	✓	✓	✓
Pensal blanco, Moll, Prensal blanco	SCL	white	Pensal Blanca	no	✓	✓	✓	✓
Quigat	E33	white	Quigat	✓	✓	✓	✓	✓
Valenci blanco *	O17	white	Beba	✓	✓	✓	✓	✓
Viñaté	E45	white	Viñaté	✓	no	✓	✓	no
Calop rojo, Calop roig	E19	rose	Beba roja	✓	✓	✓	✓	✓
Batista	E23	black	Batista	✓	✓	✓	✓	✓
Beba negra, Calop negro, Calop negre	E18	black	Valenci Tinto	✓	✓	✓	✓	✓
Boal	E21	black	Bobal	✓	✓	✓	✓	✓
Cabellis	E20	black	Manto Negro	✓	✓	✓	✓	✓
Callet	E26	black	Callet	✓	✓	✓	✓	✓
Eperó de Gall, Esperó de gall	E37	black	Eperó de gall	✓	✓	✓	✓	✓
Excursach, Escursach	E27	black	Excursach	✓	✓	✓	✓	✓
Fernandella	E22	black	Fernandella	✓	no	✓	✓	no
Fogoneu	E24	black	Fogoneu	✓	✓	✓	✓	✓
Fogoneu francés	E16	black	Fogoneu	✓	✓	✓	✓	✓
Galmete	E45	black	Mandón	✓	no	✓	✓	✓
Giró	E36	black	Mancés de Capdell	✓	✓	✓	✓	✓
Gorgollasa, Gorgollassa	E25	black	Gorgollassa	✓	✓	✓	✓	✓
Mancés de Capdell	E31	black	Mancés de Capdell	✓	✓	✓	✓	✓
Mansés de tibbus, Mancés de Tibbus	E30	black	Mansés de Tibbus	✓	✓	✓	✓	✓
Manto negro	E29	black	Manto Negro	✓	✓	✓	✓	✓
Pampolat Girat***	H24	black	Pampolat girat	✓	✓	✓	✓	✓
Sabaté, Sabater	E35	black	Sabaté	✓	✓	✓	✓	✓
Vinaté, Vinater	E11	black	Bobal	✓	✓	✓	✓	✓

[1] Ampelographic description: the varieties not described were located in a different plot where the main analysis was carried out

[2] Resistance to powdery mildew (*Erysiphe necator* Schwein.): Pensal Blanca was not analyzed since it was located in a different plot where the analysis was carried out

[3] Agronomic characterization: the varieties were not analysed since they were located in a different plot where the main analysis was carried out

4 Sensory wine analysis: vinifications were made from all the accessions of the same variety when enough grape quantity was available

Appendix II: Ampelographic descriptions and agronomic characterization

Ampelographic descriptions

Table 1. Ampelographic description per cultivar following OIV (1984) modified by Genres 081 (www.genres.de/vitis/vitis.htm). Modal data of 2006 and 2007 descriptions

Variety	Young shoot				Young leaf and shoot						Woody shoot	Flower
	OIV-001	OIV-002	OIV-003	OIV-004	OIV-007	OIV-008	OIV-015-2	OIV-016	OIV-051	OIV-053	OIV-103	OIV-151
Batista	5	2	5	5	2	2	3	1	3	5	3	3
Beba blanca	5	3	3	4	2	2	2	1	1	5	2	3
Valenci Tinto	5	2	7	3	2	2	1	1	4	1	2	3
Beba roja	5	3	5	4	2	2	1	1	3	4	2	3
Bobal	5	2	3	7	2	2	2	1	3	7	2	3
Callet	5	1	1	1	2	2	1	1	3	1	2	3
Eperó de Gall	5	3	7	5	2	2	1	1	3	6	3	3
Excursach	5	1	1	1	1	2	1	1	3	1	2	4
Fogoneu	5	2	6	1	3	2	5	1	4	1	2	3
Mansés de Capdell (Giró)	5	2	3	7	2	2	3	1	3	6	2	3
Gorgollasa	5	3	3	7	1	2	1	1	3	7	1	3
Mansés de Tibbus	5	2	7	7	2	2	2	1	4	7	2	3
Manto Negro	5	1	1	1	2	2	1	1	3	1	2	3
Pampolat girat	5	3	4	5	1	1	1	1	3	5	2	3
Pensal Blanca	5	2	3	5	1	1	1	1	2	5	1	3
Quigat	5	3	3	4	2	2	1	1	1	4	2	3
Sabaté	5	1	1	7	2	2	1	1	3	6	2	3

Variety	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
Batista	3	4	2	1	3	3	4	4	2	1	1	2	1
Beba blanca	3	3	1	2	5	5	3	5	3	2	1	3	1
Valenci Tinto	2	3	1	1	5	2	2	2	2	2	1	3	2
Beba roja	4	3	1	2	5	5	3	5	3	2	1	3	2
Bobal	4	3	1	2	5	5	3	5	2	1	1	2	2
Callet	3	3	1	1	5	4	3	3	2	1	1	3	2
Eperó de Gall	2	3	3	2	5	3	3	2	2	2	1	3	2
Excursach	2	3	1	1	5	3	4	3	2	1	1	3	3
Fogoneu	2	3	4	1	5	2	4	2	2	1	1	2	2
Mansés de Capdell (Giró)	3	3	2	1	5	3	3	2	2	1	1	2	2
Gorgollasa	2	3	1	2	5	3	2	3	2	1	1	1	3
Mansés de Tibbus	2	3	4	1	5	5	3	2	1	1	1	3	2
Manto Negro	2	3	1	1	5	3	3	2	1	2	1	3	2
Pampolat girat	3	3	1	2	3	5	2	3	2	2	1	2	2
Pensal Blanca	3	3	1	2	5	3	2	3	2	2	1	3	2
Quigat	3	3	1	1	5	3	3	3	2	2	1	3	2
Sabaté	2	3	3	1	5	5	3	2	1	1	2	3	2

Variety	Mature leaf					Bunch					
	OIV-083-2	OIV-084	OIV-087	OIV-094	OIV-306	OIV-202	OIV-203	OIV-204	OIV-206	OIV-208	OIV-209
Batista	2	4	1	7	5	2	2	7	1	1	1
Beba blanca	2	5	3	5	1	6	4	4	3	2	2
Valenci Tinto	1	1	1	3	2	6	4	3	3	2	2
Beba roja	2	3	1	5	2	6	4	3	4	2	1
Bobal	2	8	1	5	4	4	4	5	1	2	2
Callet	1	1	1	3	2	5	4	5	2	2	2
Eperó de Gall	1	7	1	5	2	5	4	7	3	2	3
Excursach	1	1	1	5	2	5	4	7	2	2	3
Fogoneu	1	1	1	5	3	5	4	6	3	2	2
Mansés de Capdell (Giró)	1	4	1	5	1	4	4	7	2	3	2
Gorgollasa	1	7	1	3	2	5	3	4	2	2	2
Mansés de Tibbus	1	7	3	5	1	6	5	5	2	2	3
Manto Negro	2	1	1	3	1	7	4	5	3	2	2
Pampolat girat	2	7	1	7	4	4	4	5	2	2	3
Pensal Blanca	2	3	1	3	1	7	5	5	4	2	2
Quigat	2	5	3	5	1	5	4	5	1	2	2
Sabaté	2	6	1	5	2	6	5	7	1	2	3

Variety	Berry										Phenology		
	OIV-220	OIV-221	OIV-223	OIV-225	OIV-230	OIV-231	OIV-232	OIV-235	OIV-236	OIV-241	OIV-301	OIV-303	OIV-304
Batista	5	5	2	5	1	1	3	1	1	3	5	3	5
Beba blanca	9	9	3	1	1	1	2	2	1	3	5	5	5
Valenci Tinto	9	7	3	5	1	1	2	2	1	3	5	5	5
Beba roja	9	7	3	2	1	1	2	2	1	3	5	7	5
Bobal	7	7	2	6	1	1	3	1	1	3	5	5	5
Callet	7	7	2	5	1	1	2	2	1	3	5	5	5
Eperó de Gall	7	7	2	5	1	1	3	1	5	3	7	7	5
Excursach	5	5	2	6	1	1	3	1	1	3	5	5	5
Fogoneu	7	7	2	6	1	1	3	1	1	3	5	5	5
Mansés de Capdell (Giró)	7	7	2	5	1	1	3	1	1	3	5	7	7
Gorgollasa	7	7	2	6	1	1	3	1	1	3	5	7	5
Mansés de Tibbus	7	7	3	5	1	1	3	1	1	3	7	7	5
Manto Negro	7	7	2	5	1	1	3	2	1	3	5	5	5
Pampolat girat	5	5	2	5	1	1	3	1	1	3	5	7	5
Pensal Blanca	7	7	2	1	1	1	3	1	1	3	5	7	7
Quigat	7	7	2	1	1	1	3	1	1	3	7	7	7
Sabaté	5	5	2	5	1	1	3	1	1	3	5	7	5

Agronomic descriptions

The following data corresponding to an agronomic characterization following OIV (1984) modified by Genres 081 (www.genres.de/vitis/vitis.htm). Data media of 2006, 2007 and 2008 vintages.

References

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

Nombre principal: Batista; Accesión: E23

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,89±0,10

Nº Racimos/Cepa: 8,46±1,66

Peso del racimo (g): 152,08±32,63

Nº Bayas/Racimo: 113,00±14,15

Peso la baya (g): 1,71±0,14

Kg uva/Cepa: 0,454±0,288

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 1,480±1,833

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,62±2,04

Peso del sarmiento (g): 30,57±5,70

kg Madera de poda/Cepa: 0,269±0,105

Caracterización del mosto:

Rto. Mosto (%): 34,37±7,78

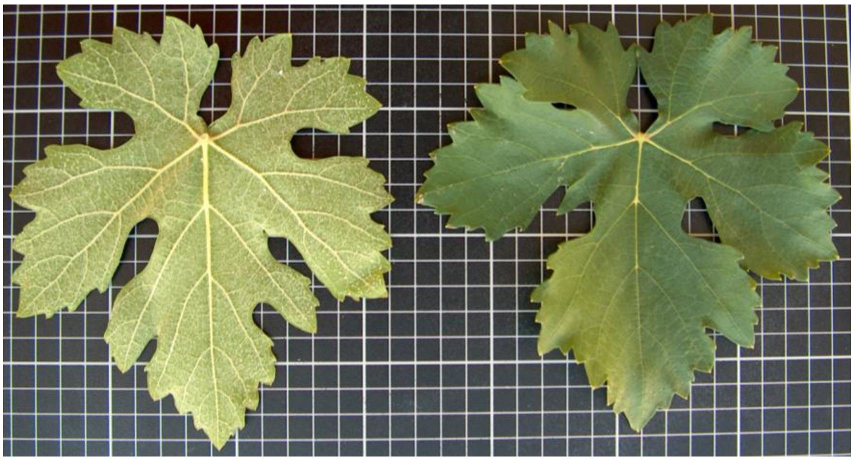
pH: 3,55±0,23

Acidez total (g/l TH_2): 5,49±0,14

%VOL: 10,91±1,29

Nota de cata:

Vino que presenta una capa media baja, de color fresa con reflejos grosellas y salmón. En nariz es un vino con intensidad media baja, destacan los aromas a frutas rojas y tonos herbáceos, con ligeros aromas cítricos y de fruta negra. En boca es un vino ligero con acidez media y bien equilibrado.



Nombre principal: Beba blanca; Accesiones: E14, E32, E39, E43, O16, O17, O44

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,75±0,08

Nº Racimos/Cepa: 6,66±1,67

Peso del racimo (g): 236,92±42,76

Nº Bayas/Racimo: 102,16±12,38

Peso la baya (g): 2,76±0,32

Kg uva/Cepa: 1,230±0,281

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,492±0,233

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,53±1,37

Peso del sarmiento (g): 52,70±6,80

kg Madera de poda/Cepa: 0,437±0,104

Caracterización del mosto:

Rto. Mosto (%): 28,28±7,56

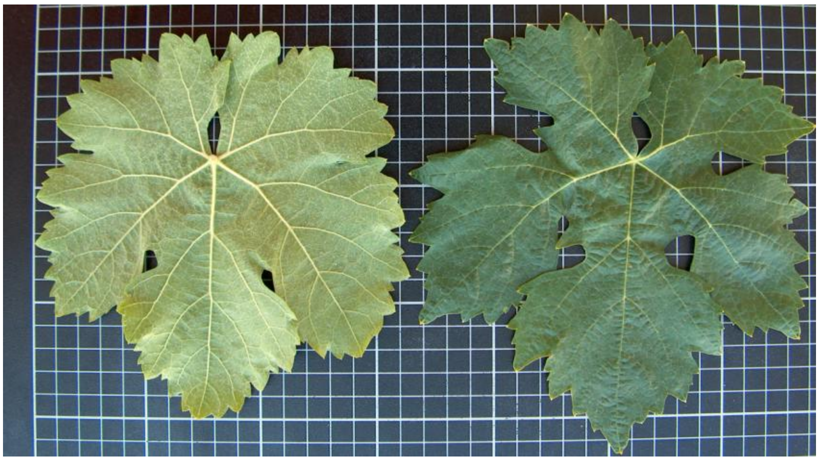
pH: 3,63±0,14

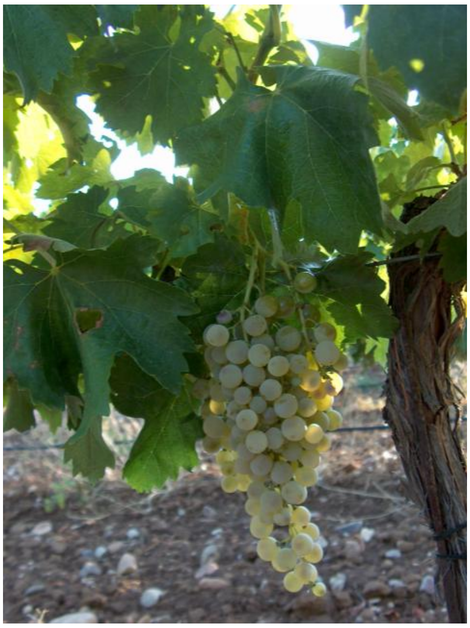
Acidez total (g/l TH_2): 4,95±0,70

%VOL: 12,72±0,07

Nota de cata:

Vino limpio y brillante de color amarillo pajizo con reflejos acerados. En nariz presenta una buena intensidad aromática. Es un vino fresco, destacan los aromas a frutas tropicales y fruta con hueso (melocotón y albaricoque). Presenta notas florales, cítricas, herbáceas y un ligero tono a pera y manzana. En boca es ligero con acidez media y bien equilibrado.





Nombre principal: Beba roja; Accesión: E19

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,88±0,14

Nº Racimos/Cepa: 6,30±1,12

Peso del racimo (g): 294,33±72,33

Nº Bayas/Racimo: 128,50±21,07

Peso la baya (g): 3,02±0,20

Kg uva/Cepa: 1,002±0,166

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 7,07±1,17

Peso del sarmiento (g): 53,82±5,98

kg Madera de poda/Cepa: 0,380±0,086

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,473±0,241

Caracterización del mosto:

Rto. Mosto (%): 28,14±7,96

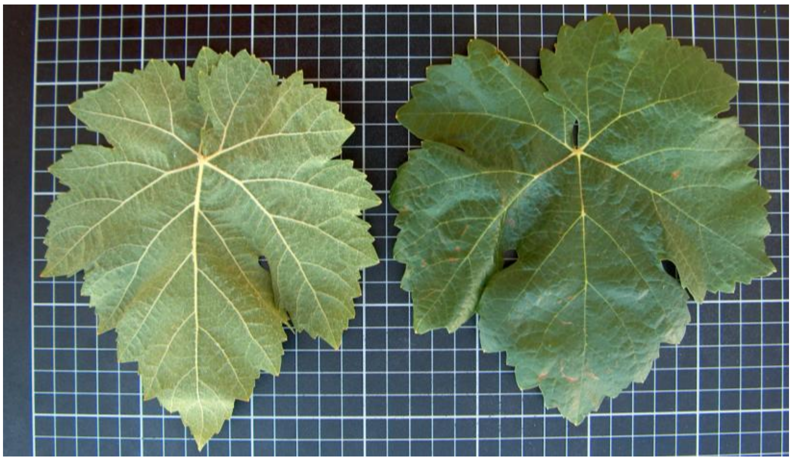
pH: 3,39±0,11

Acidez total (g/l TH_2): 5,96±0,34

%VOL: 11,45±0,65

Nota de cata:

Vino limpio y brillante de color amarillo pajizo con reflejos acerados. En nariz presenta una buena intensidad aromática. Es un vino fresco, destacan los aromas a manzana, cítricos y especiados. Presenta notas a frutas tropicales, florales y ligeros tonos a melocotón y fruta roja. En boca es un vino ligero con acidez media y bien equilibrado.



Nombre principal: Bobal; Accesiones: E11, E21

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,42±0,21

Nº Racimos/Cepa: 3,92±1,06

Peso del racimo (g): 203,27±61,04

Nº Bayas/Racimo: 116,44±27,50

Peso la baya (g): 2,41±0,57

Kg uva/Cepa: 0,608±0,341

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,862±0,872

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 9,78±1,93

Peso del sarmiento (g): 26,87±7,37

kg Madera de poda/Cepa: 0,264±0,099

Caracterización del mosto:

Rto. Mosto (%): 34,59±5,82

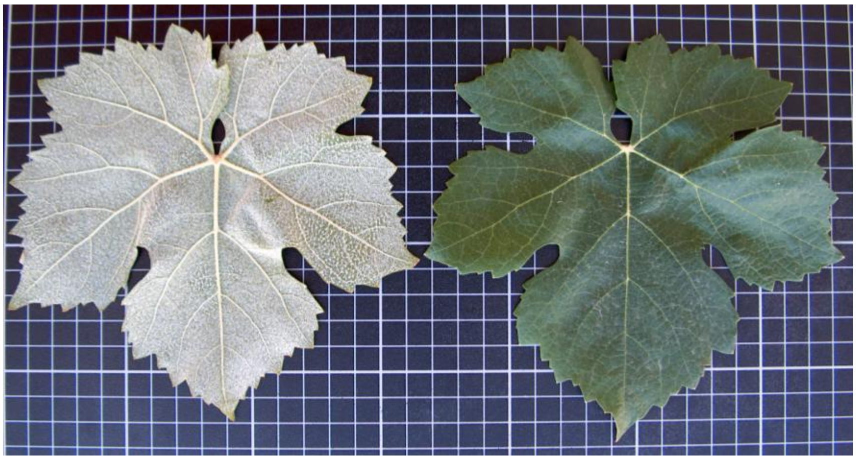
pH: 3,49±0,20

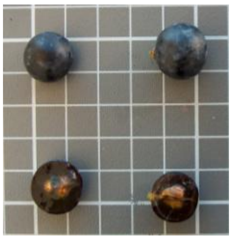
Acidez total (g/l TH_2): 6,15±0,91

%VOL: 13,82±1,10

Nota de cata:

Vino que presenta una capa alta, bien cubierto, de color púrpura con reflejos granates. En nariz es un vino con buena intensidad aromática, destacan los aromas a fruta negra y florales. Presenta tonos animales, lácticos, fruta roja y ligeros aromas herbáceos y especiados. En boca es un vino bien estructurado, con acidez media y bien equilibrado.





Nombre principal: Callet; Accesión: E26

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,89±0,29

Nº Racimos/Cepa: 8,17±2,11

Peso del racimo (g): 380,34±123,31

Nº Bayas/Racimo: 201,00±16,91

Peso la baya (g): 2,30±0,51

Kg uva/Cepa: 1,603±0,761

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,136±0,064

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 9,38±2,43

Peso del sarmiento (g): 19,41±4,09

kg Madera de poda/Cepa: 0,187±0,080

Caracterización del mosto:

Rto. Mosto (%): 32,53±6,58

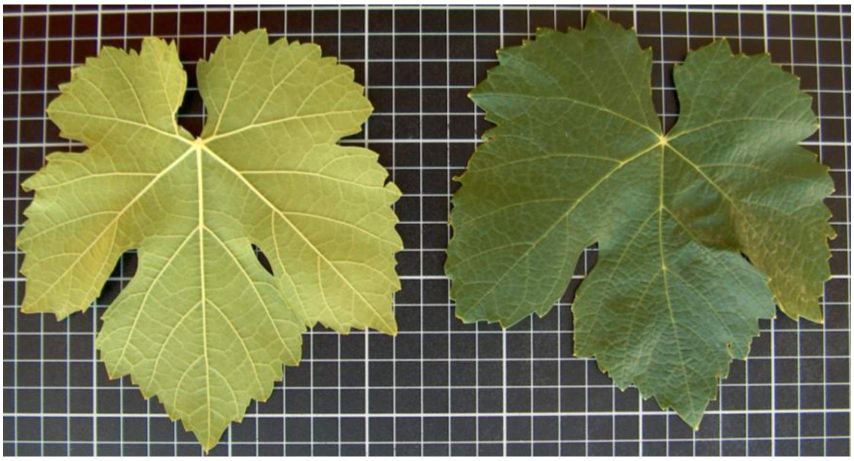
pH: 3,64±0,12

Acidez total (g/l TH_2): 5,31±0,32

%VOL: 13,07±0,36

Nota de cata:

Vino que presenta una capa media, de color rubí con reflejos granates. En nariz es un vino con buena intensidad aromática, destacan los aromas especiados, de fruta negra y roja, con tonos herbáceos. En boca es un vino de cuerpo medio, con acidez media y bien equilibrado.



Nombre principal: Eperó de Gall; Accesión: E37

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,85±0,25

Nº Racimos/Cepa: 6,93±1,06

Peso del racimo (g): 319,66±47,43

Nº Bayas/Racimo: 225,11±72,14

Peso la baya (g): 1,93±0,49

Kg uva/Cepa: 0,851±0,312

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,45±1,36

Peso del sarmiento (g): 51,89±2,97

kg Madera de poda/Cepa: 0,433±0,066

Caracterización del mosto:

Rto. Mosto (%): 32,70±8,02

pH: 3,45±0,08

Acidez total (g/l TH_2): 6,49±1,11

%VOL: 13,91±0,79

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,712±0,526

Nota de cata:

Vino que presenta una capa media alta, de color rubí con reflejos granates. En nariz es un vino con buena intensidad aromática, destacan los aromas especiados y herbáceos, que recuerdan al pimiento verde, con tonos de fruta negra y roja. En boca es un vino de cuerpo medio, con acidez media y bien equilibrado.

Nombre principal: Excursach; Accesión: E27

Parámetros de producción de vid:

Nº Racimos/Pámpano: 1,03±0,30

Nº Racimos/Cepa: 8,46±0,40

Peso del racimo (g): 224,49±182,20

Nº Bayas/Racimo: 144,94±93,55

Peso la baya (g): 1,62±0,25

Kg uva/Cepa: 0,858±0,785

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 1,187±0,960

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,55±1,83

Peso del sarmiento (g): 36,96±12,65

kg Madera de poda/Cepa: 0,329±0,150

Caracterización del mosto:

Rto. Mosto (%): 25,25±1,59

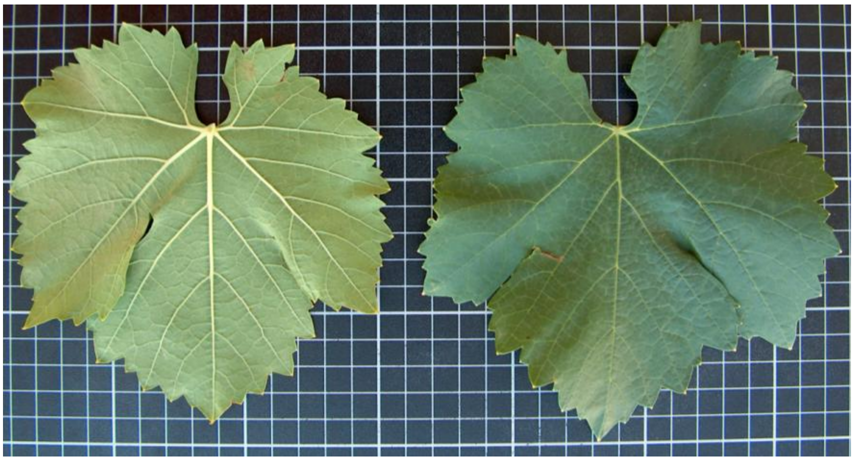
pH: 3,43±0,14

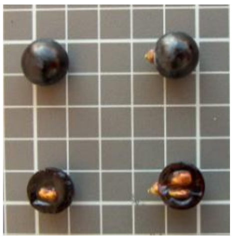
Acidez total (g/l TH_2): 6,06±2,10

%VOL: 13,77±0,81

Nota de cata:

Vino que presenta una capa alta, bien cubierto, de color púrpura con reflejos granates. En nariz es un vino con buena intensidad aromática, destacan los aromas a fruta negra, herbáceos y fruta roja, con tonos especiados, lácticos y florales. En boca es un vino bien estructurado, con acidez media y bien equilibrado.





Nombre principal: Fogoneu; Accesiones: E16, E24

Parámetros de producción de vid:

Nº Racimos/Pámpano: 1,23±0,22

Nº Racimos/Cepa: 11,18±3,00

Peso del racimo (g): 269,39±29,25

Nº Bayas/Racimo: 154,33±8,21

Peso la baya (g): 2,21±0,27

Kg uva/Cepa: 1,574±0,806

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,323±0,346

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 9,08±0,91

Peso del sarmiento (g): 28,62±3,55

kg Madera de poda/Cepa: 0,261±0,053

Caracterización del mosto:

Rto. Mosto (%): 26,49±2,91

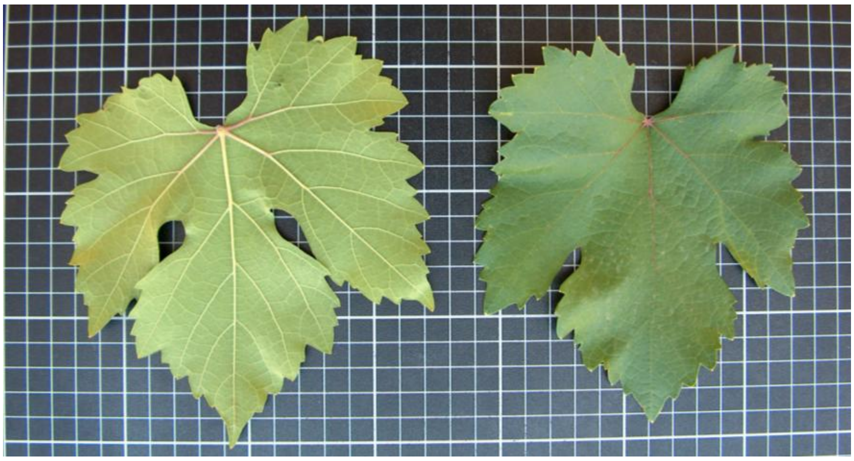
pH: 3,45±0,15

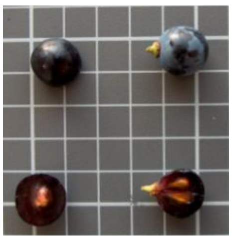
Acidez total (g/l TH_2): 6,39±1,96

%VOL: 12,41±0,36

Nota de cata:

Vino que presenta una capa alta, bien cubierto, de color granate con reflejos púrpura. En nariz presenta buena intensidad aromática destacando los aromas a frutas rojas, especiados y fruta negra. Presenta aromas herbáceos, lácticos y tonos florales. En boca es un vino bien estructurado, con acidez media y bien equilibrado.





Nombre principal: Gorgollassa; Accesión: E25

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,85±0,47

Nº Racimos/Cepa: 7,27±3,09

Peso del racimo (g): 152,25±4,48

Nº Bayas/Racimo: 97,33±29,78

Peso la baya (g): 1,92±0,58

Kg uva/Cepa: 0,595±0,461

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,815±0,602

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,40±1,38

Peso del sarmiento (g): 40,81±5,36

kg Madera de poda/Cepa: 0,347±0,097

Caracterización del mosto:

Rto. Mosto (%): 32,39±8,72

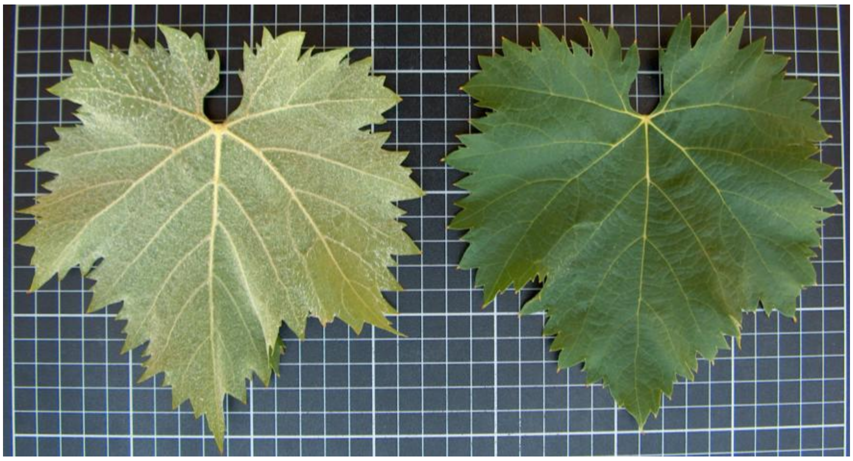
pH: 3,47±0,03

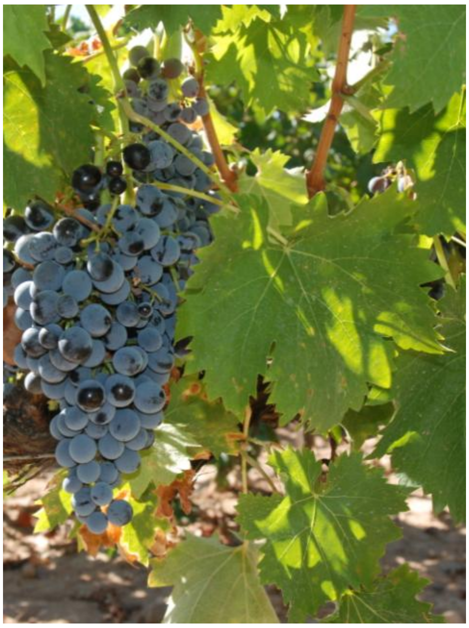
Acidez total (g/l TH_2): 5,19±0,18

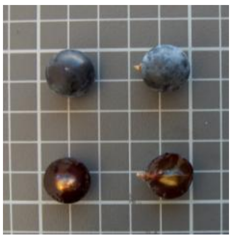
%VOL: 14,66±0,46

Nota de cata:

Vino que presenta una capa media alta, de color cereza con reflejos granates y rubí. En nariz es un vino con buena intensidad aromática, destacan los aromas florales, herbáceos y de fruta negra con tonos de frutas rojas y especiados. En boca es un vino de cuerpo medio, con acidez media y bien equilibrado.







Nombre principal: Mancés de Capell (Giró); Accesiones: E31, E36

Parámetros de producción de vid:

Nº Racimos/Pámpano: 1,14±0,31

Nº Racimos/Cepa: 10,37±1,85

Peso del racimo (g): 276,85±38,78

Nº Bayas/Racimo: 185,89±22,47

Peso la baya (g): 1,99±0,38

Kg uva/Cepa: 1,918±0,538

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,230±0,136

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,79±0,77

Peso del sarmiento (g): 43,47±18,40

kg Madera de poda/Cepa: 0,387±0,185

Caracterización del mosto:

Rto. Mosto (%): 34,48±6,04

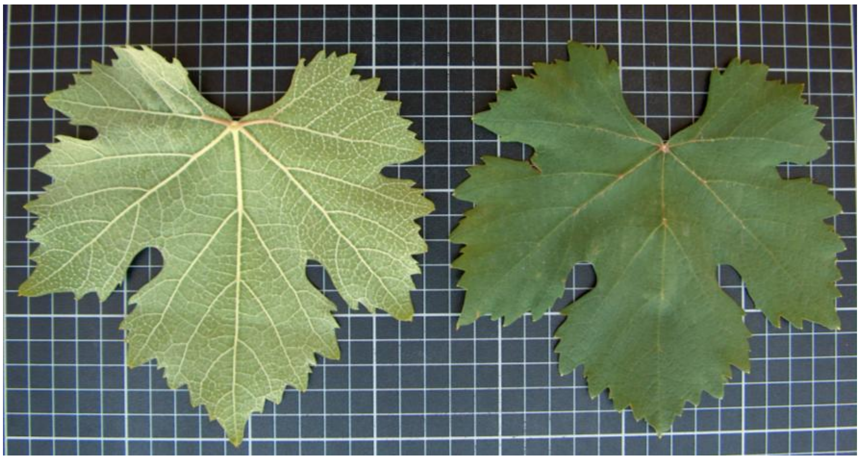
pH: 3,47±0,18

Acidez total (g/l TH_2): 4,23±0,81

%VOL: 11,36±0,76

Nota de cata:

Vino limpio y brillante de color salmón con reflejos de piel de cebolla. En nariz presenta una buena intensidad aromática, destacan los aromas a fruta negra y regaliz. Presenta notas a fruta roja, frutas tropicales y herbáceos con tonos florales. En boca es un vino ligero con acidez media baja y bien equilibrado.



Nombre principal: Mansés de Tibbus; Accesión: E30

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,92±0,25

Nº Racimos/Cepa: 7,97±1,81

Peso del racimo (g): 332,44±46,42

Nº Bayas/Racimo: 220,94±46,83

Peso la baya (g): 2,06±0,47

Kg uva/Cepa: 1,471±0,917

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,62±0,93

Peso del sarmiento (g): 58,77±1,95

kg Madera de poda/Cepa: 0,504±0,063

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,455±0,240

Caracterización del mosto:

Rto. Mosto (%): 32,84±4,50

pH: 3,60±0,16

Acidez total (g/l TH_2): 4,56±0,25

%VOL: 14,13±0,74

Nota de cata:

Vino limpio y brillante de color salmón con reflejos rosados. En nariz presenta una buena intensidad aromática, destacan los aromas a fruta con hueso y fruta negra. Presenta notas a fruta roja y frutas tropicales con tonos florales y herbáceos. En boca es un vino ligero con acidez media baja y bien equilibrado.

Nombre principal: Manto Negro; Accesión: E20, E29

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,99±0,25

Nº Racimos/Cepa: 8,78±3,03

Peso del racimo (g): 295,05±56,69

Nº Bayas/Racimo: 175,78±7,92

Peso la baya (g): 1,94±0,32

Kg uva/Cepa: 1,295±0,348

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,62±1,46

Peso del sarmiento (g): 33,73±7,68

kg Madera de poda/Cepa: 0,294±0,094

Caracterización del mosto:

Rto. Mosto (%): 27,65±8,72

pH: 3,57±0,14

Acidez total (g/l TH_2): 5,15±0,80

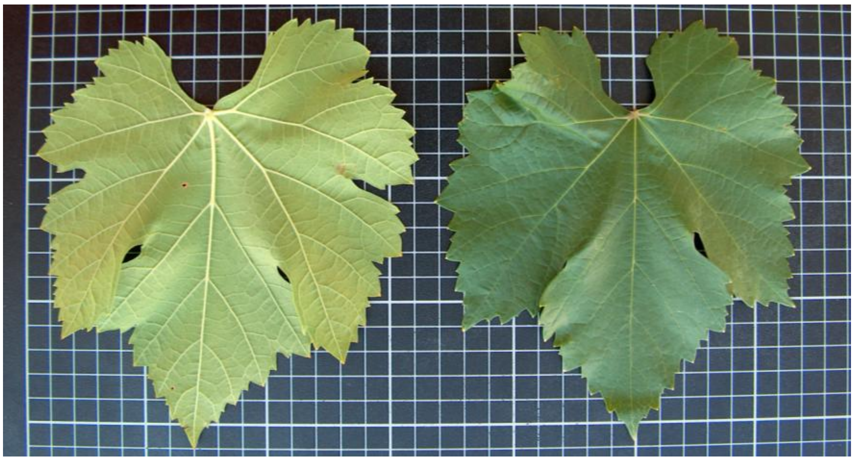
%VOL: 14,50±0,56

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,239±0,057

Nota de cata:

Vino que presenta una capa media, de color rubí con reflejos cerezas y grosellas. En nariz es un vino con buena intensidad aromática, destacan los aromas de fruta negra, florales y fruta roja, con tonos especiados y herbáceos. En boca es un vino de cuerpo medio, con acidez media y bien equilibrado.



Nombre principal: Pampolat girat; Accesión: H24

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,89±0,46

Nº Racimos/Cepa: 8,14±3,30

Peso del racimo (g): 191,25±69,26

Nº Bayas/Racimo: 145,56±16,68

Peso la baya (g): 1,48±0,50

Kg uva/Cepa: 0,858±0,427

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 9,38±2,19

Peso del sarmiento (g): 31,68±7,68

kg Madera de poda/Cepa: 0,293±0,084

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,490±0,455

Caracterización del mosto:

Rto. Mosto (%): 31,67±3,44

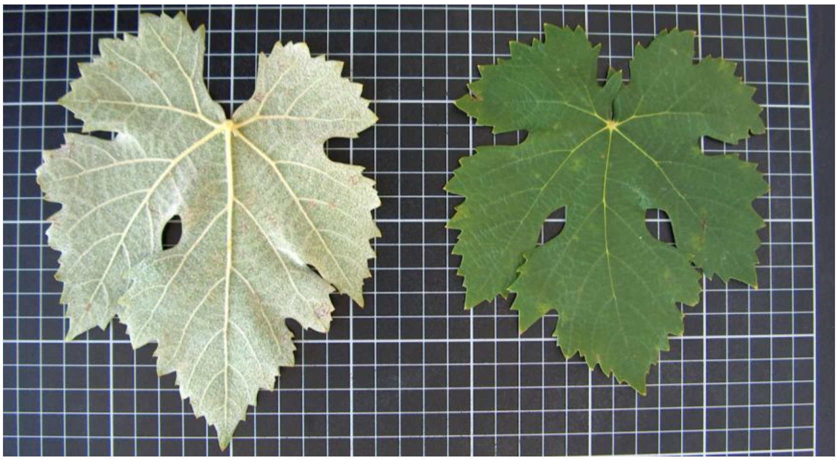
pH: 3,47±0,26

Acidez total (g/l TH_2): 5,16±1,13

%VOL: 13,63±0,81

Nota de cata:

Vino que presenta una capa media, de color cereza con reflejos rubí. En nariz es un vino de media intensidad aromática, destacan los aromas de fruta negra y roja, con tonos herbáceos y florales. En boca es un vino bien estructurado, con buena acidez y bien equilibrado.



Nombre principal: Pensal Blanca; Accesión: SCL

Parámetros de producción de vid:

Nº Racimos/Pámpano: 1,05±0,79

Nº Racimos/Cepa: 8,35±3,42

Peso del racimo (g): 448,27±84,29

Nº Bayas/Racimo: 242,28±35,18

Peso la baya (g): 2,15±0,77

Kg uva/Cepa: 1,836±1,416

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,93±2,30

Peso del sarmiento (g): 30,67±11,30

kg Madera de poda/Cepa: 0,274±0,143

Caracterización del mosto:

Rto. Mosto (%): 36,37±7,51

pH: 3,56±0,15

Acidez total (g/l TH_2): 3,63±0,78

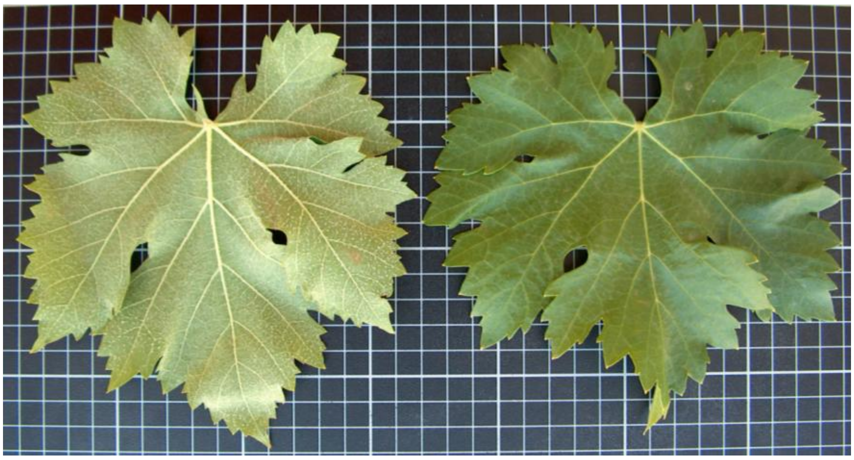
%VOL: 12,91±1,44

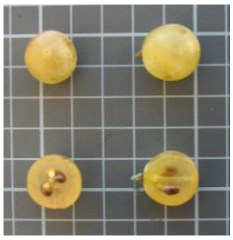
Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,232±0,159

Nota de cata:

Vino limpio y brillante de color amarillo pajizo con reflejos verdosos. En nariz presenta buena intensidad aromática. Es un vino fresco, destacan los aromas de melocotón, frutas tropicales (maracuyá, piña) y fruta verde como manzana y pera. Presenta aromas florales, herbáceas y especiadas. En boca es un vino ligero con acidez media y bien equilibrado.





Nombre principal: Quigat; Accesiones: E33, E38

Parámetros de producción de vid:

Nº Racimos/Pámpano: 1,15±0,78

Nº Racimos/Cepa: 9,07±3,78

Peso del racimo (g): 361,75±57,75

Nº Bayas/Racimo: 198,06±42,84

Peso la baya (g): 2,33±0,66

Kg uva/Cepa: 1,617±1,002

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,50±1,95

Peso del sarmiento (g): 22,39±1,08

kg Madera de poda/Cepa: 0,190±0,053

Caracterización del mosto:

Rto. Mosto (%): 26,13±9,11

pH: 3,38±0,07

Acidez total (g/l TH_2): 6,11±0,52

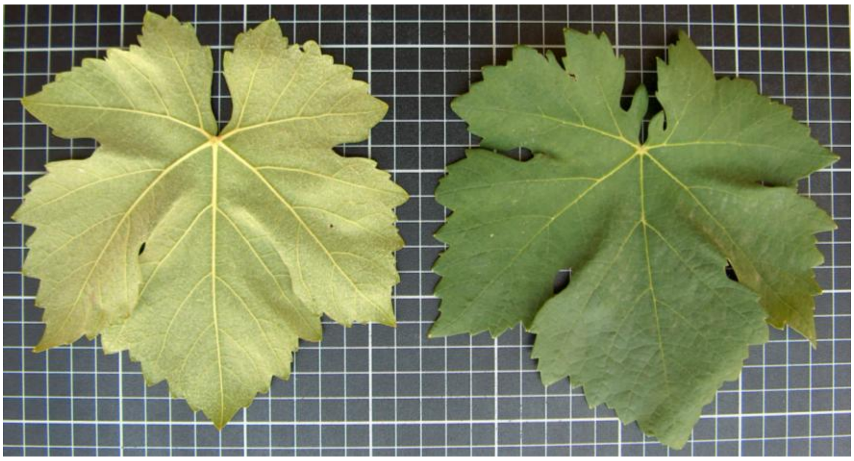
%VOL: 11,98±0,77

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,222±0,171

Nota de cata:

Vino limpio y brillante de color amarillo pajizo con reflejos verdosos. En nariz presenta una intensidad aromática media. Es un vino fresco, destacan los aromas a fruta verde, florales y herbáceos. Presenta notas a melocotón y frutas tropicales. En boca es un vino ligero con acidez media alta y bien equilibrado.



Nombre principal: Sabaté; Accesión: E35

Parámetros de producción de vid:

Nº Racimos/Pámpano: 1,10±0,27

Nº Racimos/Cepa: 9,97±2,31

Peso del racimo (g): 366,97±120,27

Nº Bayas/Racimo: 310,06±115,98

Peso la baya (g): 1,47±0,41

Kg uva/Cepa: 1,918±1,109

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 8,81±1,29

Peso del sarmiento (g): 25,66±4,13

kg Madera de poda/Cepa: 0,224±0,042

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 0,150±0,090

Caracterización del mosto:

Rto. Mosto (%): 35,08±3,99

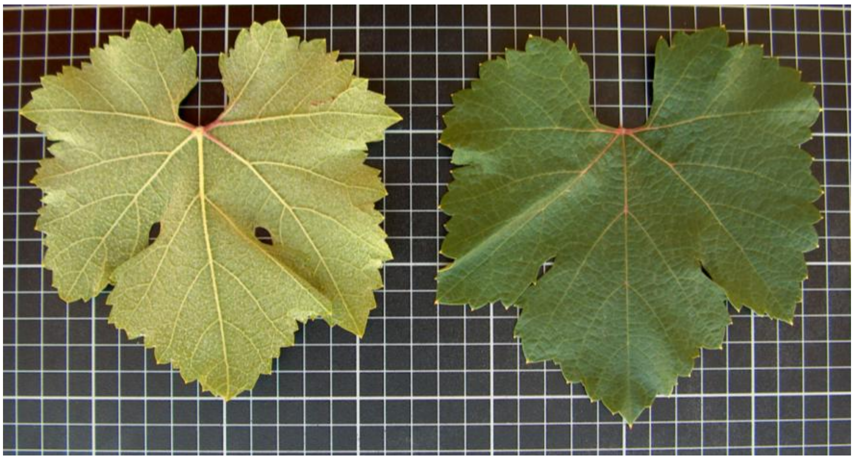
pH: 3,36±0,10

Acidez total (g/l TH_2): 6,00±0,96

%VOL: 12,36±1,26

Nota de cata:

Vino limpio y brillante de color salmón con reflejos grosellas. En nariz presenta una buena intensidad aromática, destacan los aromas a frutas con hueso y regaliz. Presenta notas cítricas, a frutas negras, frutas tropicales y florales, con tonos de fruta roja. En boca es un vino ligero con acidez media y bien equilibrado.



Nombre principal: Valenci Tinto; Accesión: E18

Parámetros de producción de vid:

Nº Racimos/Pámpano: 0,62±0,28

Nº Racimos/Cepa: 5,39±1,92

Peso del racimo (g): 203,41±95,94

Nº Bayas/Racimo: 85,39±18,76

Peso la baya (g): 3,33±0,38

Kg uva/Cepa: 0,512±0,238

Equilibrio Desarrollo/Producción:

Madera poda/Kg uva: 2,119±2,261

Parámetros de desarrollo de vid:

Número Sarmientos/Cepa: 7,78±0,79

Peso del sarmiento (g): 67,10±22,22

kg Madera de poda/Cepa: 0,536±0,218

Caracterización del mosto:

Rto. Mosto (%): 32,21±7,01

pH: 3,87±0,03

Acidez total (g/l TH_2): 4,03±0,38

%VOL: 14,22±0,44

Nota de cata:

Vino que presenta una capa media baja, de color cereza con reflejos rubí. En nariz es un vino con buena intensidad aromática, destacan los aromas a fruta roja y herbáceos con ligeros aromas a cítricos, fruta negra y tonos especiados. En boca es un vino de cuerpo medio y bien equilibrado.

OTRAS VARIEDADES ESTUDIADAS

Nombre principal: Argamusa; Accesión: E34

Parámetros de producción de vid:	Caracterización del mosto:
Peso del racimo (g): 756,40±256,40	Rto. Mosto (%): 27,09±2,26
Nº Bayas/Racimo: 276,50±46,90	pH: 3,52±0,16
Peso la baya (g): 3,05±0,72	Acidez total (g/l TH_2): 3,98±1,36
	%VOL: 11,14±0,17

Nombre principal: Fernandella; Accesión: E22

Parámetros de producción de vid:

Peso del racimo (g): 295,01±27,07

Nº Bayas/Racimo: 187,33±26,40

Peso la baya (g): 2,08±0,33

Caracterización del mosto:

Rto. Mosto (%):24,16±0,37

pH: 3,48±0,21

Acidez total (g/l TH_2): 5,40±1,97

%VOL: 14,31±0,61

Nombre principal: Mandón; Accesión: E15

Parámetros de producción de vid:

Peso del racimo (g): 366,70±31,86

Nº Bayas/Racimo: 251,83±21,92

Peso la baya (g): 1,71±0,15

Caracterización del mosto:

Rto. Mosto (%):27,19±1,37

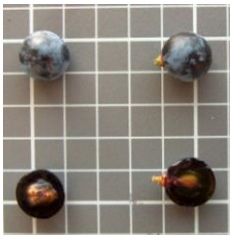
pH: 3,30±0,23

Acidez total (g/l TH_2): 6,55±2,21

%VOL: 13,70±0,63

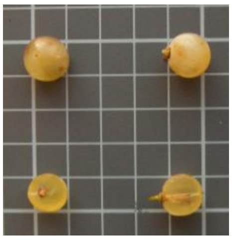
Nota de cata:

Vino que presenta una capa media, de color cereza con reflejos granates y rubí. En nariz es un vino con buena intensidad aromática, destacan los aromas de frutas rojas, lácticos y frutas negras, con tonos especiados y herbáceos. En boca es un vino de cuerpo medio, con acidez media y bien equilibrado.



Nombre principal: Viñaté; Accesión: E45

Parámetros de producción de vid:	**Caracterización del mosto:**
Peso del racimo (g): 112,33±5,03	Rto. Mosto (%): 32,55±0,72
Nº Bayas/Racimo: 129,50±2,73	pH: 3,77±0,16
Peso la baya (g): 1,29±0,13	Acidez total (g/l TH_2): 4,63±0,34
	%VOL: 13,73±1,53



Appendix III: Grape aroma data

Table 1. Compounds released by enzymatic hydrolysis from the glycosylated precursors extracted from red grapes (data are the average of the tree replicate samples; Keys in Chapter 6; Table 1). Concentrations (in micrograms per kilograms of grapes) and standard deviations. RT: Retention time; MQ (%): Match quality (%); nd: not detected

	Compounds	RT	MQ (%)	BAT.E23	BEN.E18	BOB.E11	BOB.E21	CAL.E26	EPE.E37	EXC.E27	FER.E22	FOG.E16	FOG.E24	GAL.E15	GIR.E31	GIR.E36	GOR.E25	MES.E30	MTN.E20	MTN.E29	PAM.H24	SAB.E35
1	hexanol	12.20	98	73.22 ± 14.62	67.51 ± 15.27	108.49 ± 19.78	136.71 ± 21.48	47.05 ± 7.63	100.21 ± 11.44	83.68 ± 6.51	91.38 ± 17.09	30.49 ± 9.45	29.11 ± 5.00	82.98 ± 10.78	27.01 ± 1.66	24.65 ± 2.70	91.01 ± 24.97	40.10 ± 4.60	50.11 ± 8.86	54.03 ± 8.42	66.29 ± 7.32	41.41 ± 5.94
2	cis-3-hexenol	13.53	98	17.22 ± 1.21	3.10 ± 0.61	4.37 ± 1.35	5.65 ± 1.31	2.01 ± 0.38	2.71 ± 0.56	4.78 ± 1.11	10.74 ± 1.84	5.67 ± 1.68	5.32 ± 1.13	2.90 ± 0.97	4.63 ± 0.39	4.13 ± 0.96	3.05 ± 0.4	2.12 ± 0.32	2.52 ± 0.13	3.36 ± 0.78	6.23 ± 1.73	4.21 ± 0.97
3	trans-2-hexenol	14.60	95	11.49 ± 3.65	9.53 ± 1.14	10.39 ± 1.41	16.34 ± 0.50	5.4 ± 0.56	6.28 ± 1.24	13.41 ± 1.23	10.75 ± 1.85	8.24 ± 1.20	6.63 ± 0.94	7.59 ± 0.55	4.64 ± 0.50	4.84 ± 0.64	5.6 ± 0.13	6.65 ± 1.78	8.25 ± 1.49	9.05 ± 0.99	13.77 ± 0.24	14.47 ± 1.31
4	tirosol	80.78	90	nd	nd	nd	nd	nd	nd	nd	nd	14.26 ± 0.16	nd	nd	nd	nd	nd	nd	21.16 ± 5.69	20.55 ± 6.95	nd	32.85 ± 6.87
	alcohols			**101.93 ± 18.37**	**80.14 ± 16.66**	**123.24 ± 22.54**	**158.70 ± 22.96**	**54.46 ± 8.43**	**109.21 ± 12.77**	**101.86 ± 5.15**	**112.87 ± 20.61**	**53.9 ± 13.75**	**41.06 ± 6.98**	**93.46 ± 11.69**	**36.28 ± 1.07**	**33.61 ± 3.84**	**99.66 ± 25.21**	**48.87 ± 6.68**	**82.03 ± 4.47**	**86.99 ± 13.29**	**86.30 ± 8.87**	**92.93 ± 2.36**
5	benzaldehyde	20.11	95	2.59 ± 0.28	5.51 ± 2.53	4.10 ± 1.12	6.10 ± 0.82	4.67 ± 1.38	3.87 ± 0.56	5.78 ± 1.40	3.76 ± 0.32	2.10 ± 1.22	1.97 ± 0.57	3.84 ± 0.21	2.57 ± 0.68	2.02 ± 0.41	3.91 ± 1.01	2.37 ± 0.25	3.22 ± 0.64	3.9 ± 1.00	3.81 ± 0.90	5.53 ± 1.31
6	methyl salicylate	33.38	98	11.74 ± 3.35	269.71 ± 85.63	10.56 ± 1.97	13.98 ± 2.83	20.58 ± 6.37	4.69 ± 0.91	27.18 ± 21.48	6.56 ± 1.16	5.86 ± 0.01	nd	12.36 ± 2.92	6.03 ± 2.08	4.36 ± 1.38	10.93 ± 0.97	5.47 ± 0.81	5.69 ± 1.18	4.51 ± 1.39	12.55 ± 2.32	407.40 ± 2.24
7	α-methyl-benzenemethanol	36.01	98	4.51 ± 0.63	2.19 ± 0.58	3.79 ± 0.83	7.76 ± 0.77	3.53 ± 0.06	2.92 ± 0.55	4.62 ± 0.16	4.59 ± 0.28	4.24 ± 1.21	3.01 ± 0.81	3.70 ± 0.96	4.20 ± 0.36	4.41 ± 0.74	6.60 ± 1.94	5.59 ± 0.22	4.01 ± 0.52	5.37 ± 0.96	4.29 ± 0.81	5.44 ± 0.78
8	benzyl alcohol	39.32	98	238.39 ± 48.71	557.14 ± 64.86	474.65 ± 45.14	555.67 ± 15.92	562.21 ± 99.75	339.41 ± 45.04	368.24 ± 80	579.99 ± 40.52	148.97 ± 45.55	123.25 ± 18.41	343.90 ± 47.83	164.35 ± 37.63	133.21 ± 14.09	269.39 ± 39.20	271.47 ± 25.48	329.42 ± 83.2	363.96 ± 112.12	426.23 ± 65.64	1030.05 ± 128.92
9	2-phenylethanol	41.03	98	266.6 ± 22.46	143.35 ± 14.38	203.01 ± 24.86	215.42 ± 14.09	142.57 ± 8.20	150.55 ± 18.03	194.44 ± 10.33	273.27 ± 28.62	138.90 ± 28.29	125.32 ± 19.45	114.49 ± 27.26	184.66 ± 31.53	169.22 ± 2.42	160.9 ± 18.54	187.11 ± 5.16	164.66 ± 46.64	185.65 ± 63.32	233.98 ± 14.34	295.19 ± 12.19
10	eugenol	53.55	98	14.12 ± 3.9	7.29 ± 2.39	8.28 ± 1.72	8.77 ± 1.66	nd	6.73 ± 0.42	7.87 ± 1.27	8.66 ± 0.76	nd	nd	10.42 ± 1.62	nd	nd	14.89 ± 2.85	nd	nd	nd	nd	9.05 ± 2.72
11	4-vinylguaicol	54.88	95	14.68 ± 4.19	12.63 ± 4.07	15.12 ± 7.79	14.21 ± 4.91	12.12 ± 2.37	10.03 ± 4.83	20.71 ± 6.67	8.70 ± 0.21	12.06 ± 2.10	9.68 ± 0.83	16.46 ± 3.72	11.79 ± 4.35	5.77 ± 0.14	23.65 ± 6.25	13.78 ± 2.74	10.79 ± 3.12	10.88 ± 2.68	13.10 ± 1.45	23.09 ± 6.26
12	4-vinylphenol	62.52	95	94.53 ± 46.68	28.71 ± 12.56	86.82 ± 23.87	45.61 ± 15.97	152.43 ± 7.39	68.93 ± 58.54	266.88 ± 139.27	28.85 ± 3.99	128.21 ± 5.05	153.11 ± 38.89	170.70 ± 48.55	61.33 ± 12.36	51.78 ± 10.16	67.34 ± 38.31	78.15 ± 48.39	41.78 ± 26.13	41.83 ± 13.29	91.13 ± 21.54	77.70 ± 21.12
13	methoxy eugenol	67.22	95	4.53 ± 1.43	14.08 ± 2.76	9.44 ± 2.69	6.73 ± 1.09	9.68 ± 1.42	9.02 ± 0.91	11.27 ± 2.14	5.83 ± 1.67	7.72 ± 2.26	7.81 ± 1.64	14.28 ± 2.52	nd	nd	7.01 ± 0.98	5.90 ± 1.78	12.03 ± 2.89	9.76 ± 2.63	nd	28.16 ± 8.93
14	vanillin	67.63	90	19.01 ± 1.64	21.10 ± 4.51	25.11 ± 4.09	28.08 ± 8.45	21.43 ± 7.75	33.78 ± 10.01	30.35 ± 6.20	23.46 ± 3.26	15.23 ± 4.64	13.17 ± 2.95	27.13 ± 4.53	8.92 ± 1.56	11.35 ± 3.62	36.36 ± 9.56	25.99 ± 3.06	12.47 ± 3.67	15.45 ± 1.84	24.60 ± 6.25	16.46 ± 2.31
15	methyl vanillate	68.92	95	14.65 ± 0.73	31.72 ± 7.91	35.65 ± 12.62	41.51 ± 4.84	34.71 ± 11.00	18.95 ± 4.74	35.45 ± 5.18	18.68 ± 3.18	16.83 ± 4.03	10.91 ± 0.77	25.07 ± 7.70	18.65 ± 7.98	11.32 ± 2.32	32.72 ± 12.70	21.05 ± 5.42	11.45 ± 0.45	7.09 ± 2.27	16.94 ± 0.75	18.44 ± 3.63
16	zingerone	74.20	98	15.9 ± 3.20	44.56 ± 4.6	34.48 ± 10.79	30.17 ± 1.86	37.67 ± 3.23	57.59 ± 7.35	34.50 ± 2.85	16.49 ± 3.48	11.49 ± 2.11	9.43 ± 1.44	28.07 ± 4.19	12.98 ± 1.97	15.26 ± 3.53	51.44 ± 16.26	20.48 ± 2.46	21.77 ± 1.70	17.38 ± 2.59	25.46 ± 6.49	39.01 ± 1.52
17	homovanillyl alcohol	75.71	95	23.73 ± 1.31	27.45 ± 10.28	19.76 ± 3.92	60.67 ± 15.15	12.99 ± 3.12	41.96 ± 8.33	nd	25.89 ± 6.94	17.11 ± 2.91	23.21 ± 6.47	35.57 ± 10.11	nd	16.12 ± 2.76	20.71 ± 1.54	20.35 ± 0.01	21.05 ± 1.87	16.92 ± 3.73	79.55 ± 9.72	34.37 ± 5.83
18	syringaldehyde	78.54	95	nd	nd	10.42 ± 1.54	16.19 ± 3.15	16.12 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	14.89 ± 1.47	nd	nd	8.76 ± 1.72	22.34 ± 1.65	17.08 ± 0.01
19	methyl syringate	79.25	95	32.21 ± 1.03	25.63 ± 3.93	55.69 ± 19.48	53.23 ± 13.90	34.29 ± 7.42	26.98 ± 5.54	63.18 ± 3.83	nd	52.45 ± 21.85	34.24 ± 5.03	48.89 ± 16.44	39.96 ± 15.19	28.89 ± 3.17	42.06 ± 13.22	29.70 ± 5.22	21.01 ± 4.33	21.35 ± 8.55	65.85 ± 4.28	25.57 ± 5.96
20	dihydroconiferyl alcohol	79.71	95	77.89 ± 12.59	64.88 ± 0.52	87.72 ± 28.62	43.37 ± 3.68	39.54 ± 9.37	24.20 ± 7.54	40.09 ± 21.48	53.84 ± 17.29	19.67 ± 2.10	22.05 ± 8.88	24.57 ± 5.79	33.28 ± 10.18	24.24 ± 7.69	20.71 ± 3.60	25.91 ± 6.83	36.56 ± 10.52	57.16 ± 31.53	29.72 ± 2.83	29.87 ± 7.64
	benzene compounds			**835.09 ± 113.71**	**1255.94 ± 179.66**	**1074.51 ± 91.92**	**1147.46 ± 20.46**	**1093.82 ± 135.40**	**799.61 ± 152.94**	**1110.56 ± 238.16**	**1058.58 ± 89.53**	**576.93 ± 84.39**	**537.16 ± 48.60**	**879.47 ± 163.75**	**548.69 ± 112.36**	**477.94 ± 40.56**	**778.55 ± 136.63**	**699.77 ± 38.05**	**695.90 ± 135.41**	**769.98 ± 189.52**	**1049.56 ± 90.65**	**2051.00 ± 156.21**
21	3,4-dihidro-3-oxo-a-ionol I	63.37	75	19.01 ± 3.22	19.13 ± 1.36	9.28 ± 1.78	13.76 ± 2.41	13.15 ± 3.59	11.17 ± 1.74	13.38 ± 2.39	13.49 ± 2.27	10.20 ± 0.84	9.96 ± 2.81	26.85 ± 5.52	17.79 ± 5.77	16.62 ± 3.65	23.93 ± 2.16	29.83 ± 2.84	25.06 ± 8.86	25.38 ± 7.44	24.07 ± 4.46	39.25 ± 6.95
22	3,4-dihidro-3-oxo-a-ionol II	64.38	75	30.89 ± 3.40	19.01 ± 1.87	10.36 ± 0.72	14.68 ± 0.76	15.31 ± 4.98	28.27 ± 3.52	25.21 ± 5.32	24.64 ± 3.80	18.43 ± 3.03	15.76 ± 4.10	26.16 ± 2.33	34.02 ± 8.42	31.95 ± 8.01	25.48 ± 4.16	25.62 ± 1.14	24.23 ± 4.27	21.06 ± 6.30	44.52 ± 8.53	31.46 ± 3.80
23	3,4-dihidro-3-oxo-a-ionol III	64.69	75	33.67 ± 4.78	20.40 ± 0.59	12.32 ± 0.20	16.04 ± 1.90	15.17 ± 3.48	29.49 ± 5.32	26.27 ± 4.38	23.14 ± 1.30	19.12 ± 2.21	16.30 ± 1.85	21.62 ± 3.50	30.59 ± 8.49	30.32 ± 8.29	25.11 ± 5.21	19.24 ± 1.94	22.83 ± 2.83	18.95 ± 4.48	41.68 ± 7.74	26.65 ± 1.58
24	3,4-dihidro-3-oxo-a-ionol IV	66.77	75	8.89 ± 1.98	8.44 ± 0.52	6.00 ± 1.42	10.38 ± 2.31	7.75 ± 2.42	8.92 ± 0.69	5.79 ± 1.18	4.37 ± 0.52	5.08 ± 0.82	4.02 ± 0.63	11.06 ± 2.85	4.84 ± 0.61	7.69 ± 2.14	16.27 ± 7.26	7.51 ± 1.26	8.95 ± 0.52	8.70 ± 2.73	7.68 ± 2.66	12.15 ± 0.82
25	3-hydroxy-β-damascone	66.96	95	24.34 ± 6.54	14.06 ± 2.59	8.98 ± 2.16	13.57 ± 2.49	13.72 ± 1.93	12.32 ± 2.23	26.46 ± 6.26	18.14 ± 5.78	10.94 ± 1.89	7.50 ± 0.74	22.43 ± 7.56	15.74 ± 1.79	32.68 ± 10.51	13.16 ± 3.10	12.67 ± 3.21	22.00 ± 6.78	22.67 ± 4.45	25.70 ± 0.59	18.11 ± 1.78
26	3-oxo-α-ionol	69.78	95	133.26 ± 24.41	139.42 ± 25.31	78.90 ± 16.86	87.32 ± 15.98	83.81 ± 2.27	48.78 ± 3.61	87.54 ± 9.91	107.89 ± 10.17	55.35 ± 6.99	51.39 ± 5.95	59.20 ± 17.14	158.00 ± 17.49	174.31 ± 11.75	165.64 ± 14.95	124.51 ± 13.91	87.91 ± 9.34	82.13 ± 24.52	234.07 ± 25.94	143.65 ± 5.05
27	4-oxo-α-ionol	69.98	90	22.18 ± 3.64	33.30 ± 10.77	12.70 ± 2.79	16.81 ± 4.29	14.40 ± 2.13	19.86 ± 1.22	22.57 ± 6.29	10.42 ± 2.14	16.48 ± 1.41	14.83 ± 1.41	20.33 ± 2.50	14.17 ± 1.93	16.04 ± 4.15	12.74 ± 3.54	9.74 ± 2.02	27.60 ± 4.66	24.75 ± 4.66	19.67 ± 4.15	13.48 ± 2.77

	Compounds	RT	MQ (%)	BAT.E23	BEN.E18	BOB.E11	BOB.E21	CAL.E26	EPE.E37	EXC.E27	FER.E22	FOG.E16	FOG.E24	GAL.E15	GIR.E31	GIR.E36	GOR.E25	MES.E30	MTN.E20	MTN.E29	PAM.H24	SAB.E35
28	3,9-dihydroxy-mega-5-ene	70.77	75	15.13 ± 3.25	19.90 ± 3.36	36.63 ± 3.10	35.15 ± 6.46	19.50 ± 5.03	20.79 ± 3.78	10.16 ± 0.01	17.69 ± 2.13	nd	nd	34.91 ± 10.38	16.87 ± 1.70	25.37 ± 6.93	24.72 ± 6.51	14.64 ± 1.14	52.93 ± 7.22	52.02 ± 7.81	nd	28.88 ± 1.73
29	blumenol C	71.81	75	57.73 ± 9.83	18.24 ± 4.71	44.58 ± 4.18	60.35 ± 13.11	nd	21.20 ± 3.13	25.05 ± 5.99	25.9 ± 2.74	11.25 ± 3.16	10.66 ± 2.12	13.05 ± 3.73	17.08 ± 2.78	15.64 ± 2.78	25.88 ± 5.36	10.65 ± 2.59	25.91 ± 5.55	31.18 ± 10.31	nd	6.88 ± 1.81
30	5,6-epoxy-trans-β-ionone	72.38	75	7.87 ± 1.56	12.10 ± 3.69	9.03 ± 1.91	20.93 ± 4.91	8.48 ± 2.01	12.29 ± 3.24	9.08 ± 2.52	7.43 ± 1.17	2.89 ± 0.95	6.33 ± 1.89	nd	9.42 ± 1.86	9.22 ± 2.94	7.38 ± 1.78	9.02 ± 1.18	7.48 ± 1.09	3.39 ± 0.70	8.68 ± 2.13	nd
31	3-hydroxy-7,8-dihydro-β-ionol	73.41	75	26.74 ± 6.82	31.00 ± 11.43	15.94 ± 5.10	17.93 ± 1.97	21.49 ± 4.05	17.03 ± 2.73	22.97 ± 4.9	35.49 ± 5.58	14.05 ± 1.94	24.25 ± 1.64	32.15 ± 7.37	24.71 ± 3.97	33.68 ± 4.41	27.34 ± 4.11	26.40 ± 2.81	36.14 ± 3.98	30.31 ± 6.14	42.00 ± 10.25	31.75 ± 1.98
32	vomifoliol	85.91	85	274.23 ± 20.16	274.49 ± 62.46	116.84 ± 20.98	163.37 ± 17.78	104.73 ± 22.40	112.57 ± 8.97	113.91 ± 23.42	174.06 ± 41.14	74.99 ± 24.61	75.12 ± 15.16	95.13 ± 25.12	230.06 ± 67	193.32 ± 37.59	97.91 ± 16.42	218.93 ± 21.56	141.14 ± 40.71	173.38 ± 43.15	204.00 ± 9.20	297.27 ± 42.80
	norisoprenoids			**653.93 ± 53.31**	**609.49 ± 107.45**	**361.55 ± 46.3**	**470.29 ± 27.43**	**317.52 ± 36.24**	**342.69 ± 14.52**	**381.62 ± 26.52**	**462.67 ± 55.91**	**238.78 ± 43.92**	**236.11 ± 10.34**	**362.9 ± 70.55**	**573.29 ± 111.18**	**586.84 ± 62.41**	**457.33 ± 34.27**	**508.76 ± 8.63**	**482.18 ± 54.69**	**493.91 ± 98.48**	**652.06 ± 64.38**	**647.25 ± 46.49**
33	trans-furan linalool oxide	16.07	90	17.23 ± 3.72	8.50 ± 1.24	2.01 ± 0.70	1.17 ± 0.25	2.63 ± 0.44	7.94 ± 1.79	11.67 ± 1.01	8.76 ± 0.94	7.47 ± 0.80	5.62 ± 1.21	7.89 ± 1.57	19.40 ± 3.46	18.15 ± 2.10	16.58 ± 6.12	2.12 ± 0.31	5.47 ± 0.45	5.59 ± 0.90	8.34 ± 2.55	2.57 ± 0.34
34	cis-furan linalool oxide	17.51	90	18.06 ± 3.38	8.73 ± 1.59	2.32 ± 0.44	0.96 ± 0.14	3.29 ± 0.70	5.76 ± 0.68	16.22 ± 0.53	14.88 ± 1.55	7.75 ± 1.14	4.30 ± 0.26	8.14 ± 2.23	5.00 ± 1.10	7.77 ± 1.67	10.03 ± 5.68	4.10 ± 1.29	5.78 ± 1.77	6.95 ± 1.11	8.30 ± 1.12	4.79 ± 1.15
35	linalool	21.91	98	3.94 ± 0.01	nd	0.41 ± 0.17	0.63 ± 0.05	nd	nd	nd	nd	2.76 ± 0.90	nd	nd	29.68 ± 5.77	60.16 ± 7.54	nd	nd	4.2 ± 0.01	4.14 ± 0.01	10.09 ± 2.19	nd
36	α-terpineol	29.65	98	16.55 ± 3.68	11.81 ± 2.33	nd	nd	6.37 ± 1.33	nd	6.08 ± 1.11	2.20 ± 0.15	4.64 ± 1.29	6.41 ± 0.81	3.33 ± 1.06	24.81 ± 2.21	26.7 ± 2.86	45.19 ± 9.79	13.89 ± 2.50	5.08 ± 1.52	5.81 ± 0.82	31.33 ± 4.18	nd
37	trans-pyran linalool oxide	31.93	75	20.08 ± 3.20	13.15 ± 1.96	1.51 ± 0.18	nd	5.16 ± 0.73	17.10 ± 3.67	27.46 ± 2.83	19.43 ± 3.89	16.13 ± 3.30	10.28 ± 2.10	17.63 ± 4.32	8.93 ± 1.46	11.53 ± 1.73	32.21 ± 10.35	nd	8.12 ± 1.76	7.04 ± 2.19	11.77 ± 0.79	nd
38	cis-pyran linalool oxide	33.44	75	13.76 ± 3.57	3.63 ± 0.50	1.99 ± 0.08	nd	4.26 ± 1.36	3.05 ± 0.93	6.73 ± 0.23	9.32 ± 1.94	8.06 ± 0.69	4.22 ± 0.71	5.38 ± 1.47	3.94 ± 0.32	5.70 ± 1.21	5.75 ± 1.63	nd	2.80 ± 0.33	2.37 ± 0.71	6.04 ± 0.56	nd
39	citronellol	33.85	80	nd	nd	1.42 ± 0.19	2.34 ± 0.61	3.47 ± 0.16	3.99 ± 1.09	4.78 ± 0.28	8.22 ± 2.63	5.13 ± 2.24	2.07 ± 0.01	nd	4.83 ± 0.87	2.24 ± 0.01	nd	nd	nd	nd	nd	nd
40	nerol	35.51	98	42.82 ± 5.19	6.20 ± 1.55	10.16 ± 3.54	12.78 ± 1.29	9.34 ± 2.85	10.60 ± 1.21	8.98 ± 1.47	34.93 ± 4.45	5.80 ± 3.27	3.46 ± 0.35	10.29 ± 1.30	9.04 ± 3.20	8.39 ± 0.61	9.35 ± 2.50	12.94 ± 3.76	7.89 ± 2.44	9.40 ± 3.05	23.61 ± 5.13	6.59 ± 0.09
41	geraniol	38.18	98	108.6 ± 11.22	18.92 ± 4.24	25.99 ± 2.89	30.23 ± 1.83	20.07 ± 2.05	20.87 ± 4.84	19.09 ± 3.19	51.3 ± 3.09	17.59 ± 0.40	14.42 ± 2.41	12.21 ± 3.90	42.02 ± 4.39	54.87 ± 5.41	19.50 ± 5.84	58.54 ± 7.32	17.58 ± 4.71	18.71 ± 1.89	111.29 ± 13.04	15.68 ± 1.22
42	diol (2,6-dimetil-3,7-ottadien-2,6-diolo)	43.52	95	13.58 ± 4.67	5.43 ± 0.57	nd	nd	nd	4.85 ± 0.95	6.46 ± 0.22	6.9 ± 1.27	nd	nd	5.79 ± 1.64	9.92 ± 1.18	13.3 ± 2.99	9.47 ± 0.30	nd	nd	nd	5.17 ± 1.09	nd
43	endiol	45.17	75	5.54 ± 1.02	nd	nd	nd	nd	nd	nd	4.04 ± 0.80	nd	nd	3.20 ± 1.22	nd	nd	nd	3.97 ± 0.01	nd	nd	nd	nd
44	cresol	46.04	90	nd	nd	nd	1.78 ± 0.05	1.70 ± 0.35	nd	nd	nd	1.50 ± 0.25	1.16 ± 0.35	2.86 ± 0.88	nd	nd	nd	nd	nd	nd	nd	nd
45	hydroxy citronellol	55.89	75	nd	nd	nd	nd	6.08 ± 1.62	9.39 ± 3.17	9.01 ± 0.37	6.21 ± 1.39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
46	8-hydroxydihydrolinalool	56.06	75	3.71 ± 0.88	9.31 ± 1.99	nd	nd	4.32 ± 1.06	9.21 ± 2.58	3.86 ± 0.40	8.14 ± 1.30	3.49 ± 0.82	2.88 ± 0.22	4.15 ± 1.37	11.25 ± 3.22	7.4 ± 0.49	11.04 ± 3.43	nd	8.18 ± 1.57	8.57 ± 1.51	10.20 ± 0.74	9.98 ± 2.64
47	trans-8-hydroxy-linalool	58.52	90	51.39 ± 7.48	112.25 ± 9.98	9.47 ± 3.13	5.43 ± 1.03	6.85 ± 0.95	43.25 ± 3.85	18.42 ± 3.7	43.99 ± 5.08	19.68 ± 3.39	15.87 ± 0.20	14.89 ± 1.74	24.24 ± 6.27	26.52 ± 7.26	36.74 ± 1.61	9.03 ± 0.57	23.82 ± 6.68	31.78 ± 7.72	24.98 ± 4.43	29.45 ± 0.77
48	hydroxy geraniol	59.94	75	16.60 ± 12.13	3.88 ± 0.56	4.71 ± 3.60	2.85 ± 0.58	3.80 ± 0.70	6.06 ± 0.82	3.90 ± 0.81	7.71 ± 1.38	3.36 ± 0.96	3.58 ± 0.32	3.32 ± 1.18	19.7 ± 5.9	16.23 ± 3.41	5.33 ± 0.34	10.18 ± 1.19	3.61 ± 0.37	4.18 ± 0.76	9.14 ± 2.91	5.90 ± 1.05
49	cis-8-hydroxy-linalool	59.97	75	56.75 ± 17.08	42.60 ± 3.38	15.67 ± 2.33	16.31 ± 1.05	30.30 ± 4.11	71.07 ± 5.65	18.23 ± 3.20	48.08 ± 4.47	36.24 ± 8.72	30.37 ± 3.78	16.66 ± 2.03	245.92 ± 47.64	257.40 ± 50.66	46.45 ± 3.55	34.91 ± 9.78	22.65 ± 3.48	21.46 ± 2.53	71.99 ± 15.70	31.71 ± 3.41
50	geranic acid	60.67	95	54.19 ± 5.30	26.37 ± 4.05	13.80 ± 2.58	16.57 ± 3.82	21.44 ± 5.50	55.61 ± 2.02	36.71 ± 6.19	24.34 ± 2.01	17.48 ± 3.90	18.51 ± 4.38	49.48 ± 10.41	55.35 ± 11.68	69.55 ± 7.38	44.04 ± 2.21	26.67 ± 1.93	15.49 ± 2.33	17.55 ± 1.44	99.45 ± 12.08	13.27 ± 2.77
51	p-menthene-7,8-diol	66.67	95	125.76 ± 34.78	55.37 ± 2.29	nd	10.13 ± 2.03	14.69 ± 3.36	9.77 ± 1.74	nd	nd	6.55 ± 0.72	11.27 ± 2.50	13.81 ± 4.04	45.63 ± 4.88	44.73 ± 12.62	26.29 ± 0.66	43.9 ± 3.46	22.61 ± 5.28	18.31 ± 5.31	98.97 ± 24.38	nd
	terpenes			**565.92 ± 111.15**	**326.15 ± 27.87**	**87.68 ± 11.53**	**95.52 ± 8.02**	**143.76 ± 8.66**	**275.26 ± 32.25**	**192.99 ± 4.53**	**296.40 ± 11.87**	**158.30 ± 14.87**	**133.03 ± 1.07**	**174.42 ± 19.48**	**559.66 ± 72.83**	**629.16 ± 74.58**	**317.99 ± 39.97**	**217.61 ± 9.36**	**150.47 ± 20.63**	**159.08 ± 10.95**	**530.67 ± 80.09**	**119.92 ± 3.64**
	Total			**2156.88 ± 244.83**	**2271.72 ± 271.62**	**1646.99 ± 124.62**	**1871.97 ± 71.61**	**1609.56 ± 169.03**	**1526.78 ± 182.73**	**1787.03 ± 222.15**	**1930.52 ± 176.07**	**1027.92 ± 145.08**	**947.35 ± 63.60**	**1510.24 ± 263.48**	**1717.93 ± 267.17**	**1727.56 ± 136.08**	**1653.53 ± 211.08**	**1475.01 ± 37.66**	**1410.58 ± 207.76**	**1509.96 ± 288.26**	**2318.58 ± 201.84**	**2911.11 ± 201.02**

Table 2 Compounds released by enzymatic hydrolysis from the glycosylated precursors extracted from white and rose grapes (data are the average of the tree replicate samples; Keys in Chapter 6; Table 1). Concentrations (in micrograms per kilograms of grapes) and standard deviations. RT: Retention time; MQ (%): Match quality (%); nd: not detected

	Compounds	RT	MQ (%)	ARG.E34	BEB.E14	BEB.E32	BEB.E39	BEB.E43	BEB.O16	BEB.O17	BEB.O44	BER.E19	PEN.SC	QUI.E33	QUI.E38	VIN.E45
1	hexanol	12.20	98	32.03 ± 2.52	31.18 ± 1.57	22.23 ± 0.38	34.06 ± 3.46	27.50 ± 3.47	29.59 ± 4.80	29.73 ± 4.45	44.86 ± 2.51	33.93 ± 4.56	27.79 ± 2.28	56.66 ± 4.13	49.96 ± 7.46	55.39 ± 6.82
2	cis-3-hexenol	13.53	98	6.99 ± 0.55	2.32 ± 0.57	2.03 ± 0.17	1.91 ± 0.29	2.60 ± 0.61	2.32 ± 0.34	2.39 ± 0.74	1.33 ± 0.15	3.60 ± 0.77	15.52 ± 1.14	1.99 ± 0.25	2.66 ± 0.56	9.42 ± 0.61
3	trans-2-hexenol	14.60	95	10.58 ± 1.77	6.14 ± 0.96	7.48 ± 1.12	3.96 ± 0.23	8.16 ± 0.68	12.61 ± 2.01	8.94 ± 1.06	5.03 ± 0.16	10.41 ± 2.70	14.90 ± 0.97	3.97 ± 0.76	3.03 ± 0.64	7.72 ± 0.06
4	tirosol	80.78	90	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	alcohols			**49.60 ± 2.70**	**39.64 ± 2.91**	**31.75 ± 1.46**	**39.93 ± 3.40**	**38.26 ± 4.65**	**44.52 ± 6.75**	**41.06 ± 6.25**	**51.22 ± 2.56**	**47.94 ± 3.51**	**58.20 ± 2.79**	**62.63 ± 4.85**	**55.65 ± 7.82**	**72.53 ± 7.24**
5	benzaldehyde	20.11	95	1.99 ± 0.39	4.16 ± 1.66	2.29 ± 0.26	3.06 ± 1.06	2.10 ± 0.50	2.18 ± 0.68	5.39 ± 4.09	3.13 ± 0.88	nd	2.38 ± 0.20	2.26 ± 0.46	3.45 ± 0.93	140.66 ± 4.02
6	methyl salicylate	33.38	98	10.04 ± 3.08	17.56 ± 7.77	23.4 ± 6.79	nd	10.28 ± 6.47	19.96 ± 10.10	48.72 ± 36.94	4.02 ± 0.24	17.19 ± 6.77	4.46 ± 0.48	7.41 ± 1.63	7.57 ± 0.93	10.32 ± 1.50
7	α-methyl-benzenemethanol	36.01	98	3.15 ± 0.77	2.98 ± 0.47	3.57 ± 0.58	2.89 ± 0.38	3.63 ± 0.39	3.95 ± 0.25	3.30 ± 0.90	2.86 ± 0.82	4.89 ± 2.59	2.76 ± 0.29	3.23 ± 0.15	3.72 ± 0.45	7.03 ± 0.45
8	benzyl alcohol	39.32	98	180.07 ± 10.84	212.03 ± 29.01	291.17 ± 33.75	211.49 ± 20.76	122.86 ± 12.17	283.61 ± 27.78	237.99 ± 25.57	154.61 ± 24.17	191.18 ± 57.60	167.53 ± 5.99	298.64 ± 21.86	418.50 ± 77.16	536.78 ± 14.65
9	2-phenylethanol	41.03	98	187.23 ± 21.57	243.92 ± 27.30	268.58 ± 32.52	214.70 ± 14.09	296.23 ± 31.87	270.81 ± 30.99	245.23 ± 47.59	183.67 ± 12.85	257.77 ± 52.33	120.32 ± 5.53	258.82 ± 12.68	271.72 ± 4.63	306.42 ± 18.12
10	eugenol	53.55	98	5.55 ± 1.64	3.16 ± 0.52	6.30 ± 1.22	5.12 ± 1.65	2.69 ± 0.92	5.44 ± 1.06	6.51 ± 2.33	3.50 ± 0.85	5.19 ± 1.74	3.72 ± 0.14	6.08 ± 0.89	5.84 ± 1.19	15.97 ± 2.69
11	4-vinylguaicol	54.88	95	9.87 ± 1.85	10.51 ± 2.43	7.97 ± 0.34	9.34 ± 0.46	9.64 ± 1.59	11.73 ± 2.24	12.41 ± 4.11	11.68 ± 2.30	10.11 ± 0.33	16.75 ± 1.01	23.14 ± 4.52	22.61 ± 2.55	22.65 ± 3.25
12	4-vinylphenol	62.52	95	13.11 ± 2.42	18.73 ± 4.97	7.96 ± 1.33	7.56 ± 0.58	13.29 ± 0.85	11.84 ± 3.64	9.62 ± 2.79	17.66 ± 7.79	10.14 ± 1.57	4.79 ± 1.36	21.38 ± 4.67	29.31 ± 6.25	15.76 ± 1.37
13	methoxy eugenol	67.22	95	5.24 ± 1.12	4.58 ± 1.45	8.90 ± 1.81	4.28 ± 0.39	5.89 ± 1.35	7.66 ± 0.37	8.05 ± 0.92	3.14 ± 0.01	8.22 ± 3.44	nd	6.34 ± 1.28	2.99 ± 0.25	40.48 ± 2.50
14	vanillin	67.63	90	8.83 ± 0.98	10.02 ± 4.41	16.27 ± 5.09	24.19 ± 10.11	14.77 ± 4.46	13.73 ± 4.12	20.75 ± 6.53	11.77 ± 2.25	12.42 ± 1.58	16.18 ± 4.22	13.31 ± 2.20	17.70 ± 5.47	10.24 ± 1.25
15	methyl vanillate	68.92	95	nd	nd	4.49 ± 0.01	nd	nd	nd	nd	nd	nd	7.56 ± 1.95	11.50 ± 2.72	10.78 ± 1.79	8.90 ± 0.62
16	zingerone	74.20	98	18.09 ± 1.85	29.82 ± 0.79	15.62 ± 0.84	15.22 ± 1.53	25.81 ± 5.77	12.30 ± 3.46	16.95 ± 4.55	26.10 ± 2.56	27.49 ± 3.98	17.89 ± 1.16	17.17 ± 0.74	22.14 ± 0.24	42.10 ± 2.40
17	homovanillyl alcohol	75.71	95	35.03 ± 15.16	27.25 ± 8.66	12.74 ± 3.78	10.57 ± 0.92	31.98 ± 2.59	13.01 ± 2.78	15.21 ± 2.62	17.59 ± 2.62	32.92 ± 0.82	37.27 ± 3.22	46.30 ± 13.55	40.60 ± 11.56	35.66 ± 5.93
18	syringaldehyde	78.54	95	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	19.21 ± 1.67
19	methyl syringate	79.25	95	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	19.83 ± 3.83	17.87 ± 4.68	nd
20	dihydroconiferyl alcohol	79.71	95	56.75 ± 24.26	41.09 ± 6.92	46.77 ± 4.75	57.02 ± 18.43	76.05 ± 24.42	63.15 ± 18.95	48.99 ± 3.95	62.80 ± 27.99	49.66 ± 15.13	104.12 ± 13.26	49.31 ± 4.63	44.12 ± 7.07	30.09 ± 4.25
	benzene compounds			**534.97 ± 32.23**	**625.80 ± 70.23**	**713.03 ± 72.47**	**565.44 ± 49.83**	**614.33 ± 53.47**	**715.02 ± 10.75**	**679.10 ± 118.70**	**495.18 ± 69.21**	**627.19 ± 109.95**	**505.70 ± 14.70**	**784.73 ± 14.12**	**918.92 ± 103.31**	**1242.26 ± 12.48**
21	3,4-dihidro-3-oxo-a-ionol I	63.37	75	19.36 ± 4.49	16.61 ± 1.47	20.46 ± 6.59	18.10 ± 2.33	21.85 ± 4.14	15.46 ± 2.49	22.76 ± 2.66	20.21 ± 5.67	19.62 ± 4.81	8.60 ± 1.59	14.52 ± 1.64	19.27 ± 3.59	20.03 ± 1.98
22	3,4-dihidro-3-oxo-a-ionol II	64.38	75	18.69 ± 3.76	18.91 ± 1.58	19.26 ± 6.30	15.67 ± 1.98	25.54 ± 2.45	14.05 ± 2.54	20.88 ± 5.10	23.26 ± 4.91	20.73 ± 2.18	7.36 ± 0.37	22.96 ± 1.33	31.36 ± 5.83	36.28 ± 5.65
23	3,4-dihidro-3-oxo-a-ionol III	64.69	75	17.78 ± 3.41	22.09 ± 1.26	19.74 ± 3.77	17.94 ± 1.34	29.82 ± 4.08	16.57 ± 2.50	23.92 ± 4.23	22.21 ± 2.80	25.06 ± 1.33	12.10 ± 0.34	22.62 ± 2.47	28.24 ± 3.76	36.55 ± 3.09
24	3,4-dihidro-3-oxo-a-ionol IV	66.77	75	7.16 ± 0.77	5.36 ± 0.26	6.00 ± 1.33	7.62 ± 0.55	13.84 ± 2.37	7.43 ± 1.34	10.79 ± 1.66	7.87 ± 1.09	12.80 ± 0.94	4.23 ± 0.78	6.31 ± 1.57	10.67 ± 3.18	11.64 ± 1.18
25	3-hydroxy-β-damascone	66.96	95	8.73 ± 1.67	12.31 ± 2.32	10.28 ± 0.85	10.90 ± 2.01	20.47 ± 6.09	10.66 ± 4.06	12.52 ± 1.58	10.75 ± 2.30	16.52 ± 4.68	nd	26.77 ± 3.74	24.55 ± 4.99	26.65 ± 2.96
26	3-oxo-α-ionol	69.78	95	78.72 ± 11.85	193.44 ± 13.43	209.96 ± 53.23	166.20 ± 16.75	192.96 ± 34.97	181.38 ± 32.53	183.69 ± 24.87	183.55 ± 26.26	171.19 ± 14.69	20.65 ± 2.15	127.11 ± 13.18	126.26 ± 5.98	123.62 ± 22.81
27	4-oxo-α-ionol	69.98	90	16.34 ± 2.39	21.19 ± 2.73	17.04 ± 3.40	13.98 ± 1.84	19.43 ± 3.72	17.25 ± 1.87	15.97 ± 0.22	18.00 ± 2.24	11.29 ± 3.42	7.00 ± 1.99	18.83 ± 3.17	16.44 ± 1.70	29.66 ± 9.69

	Compounds	RT	MQ (%)	ARG.E34	BEB.E14	BEB.E32	BEB.E39	BEB.E43	BEB.O16	BEB.O17	BEB.O44	BER.E19	PEN.SC	QUI.E33	QUI.E38	VIN.E45
28	3,9-dihydroxy-mega-5-ene	70.77	75	22.47 ± 3.97	35.49 ± 0.65	17.39 ± 4.44	23.76 ± 0.59	40.17 ± 6.22	12.67 ± 1.02	28.20 ± 1.15	39.12 ± 5.12	32.01 ± 6.26	6.94 ± 1.26	49.66 ± 6.59	42.34 ± 1.54	55.20 ± 11.77
29	blumenol C	71.81	75	7.44 ± 1.08	14.20 ± 1.26	12.73 ± 2.57	10.07 ± 1.97	25.91 ± 4.73	9.21 ± 2.21	15.52 ± 3.96	20.04 ± 4.87	18.30 ± 2.81	10.98 ± 2.13	42.26 ± 4.14	39.34 ± 0.97	36.23 ± 6.29
30	5,6-epoxy-trans-β-ionone	72.38	75	2.65 ± 0.52	6.84 ± 1.30	6.16 ± 1.32	7.84 ± 2.25	6.77 ± 1.25	6.68 ± 1.45	9.45 ± 1.99	7.92 ± 2.14	10.62 ± 2.71	nd	9.02 ± 2.09	10.92 ± 4.86	33.21 ± 5.32
31	3-hydroxy-7,8-dihydro-β-ionol	73.41	75	14.76 ± 0.49	12.32 ± 2.28	19.53 ± 3.99	19.04 ± 5.76	33.13 ± 7.78	23.74 ± 7.54	21.05 ± 3.17	20.01 ± 1.64	25.37 ± 7.93	8.63 ± 1.06	36.07 ± 3.72	33.69 ± 3.10	42.52 ± 8.11
32	vomifoliol	85.91	85	170.03 ± 51.81	165.84 ± 21.62	188.33 ± 32.33	151.17 ± 48.86	296.68 ± 44.60	234.41 ± 26.30	240.48 ± 71.05	159.13 ± 25.99	268.38 ± 55.08	269.42 ± 12.16	279.07 ± 39.09	234.18 ± 40.54	249.79 ± 24.99
	norisoprenoids			**384.14 ± 74.68**	**524.61 ± 37.54**	**546.88 ± 101.27**	**462.28 ± 57.33**	**726.57 ± 73.53**	**549.50 ± 54.47**	**605.24 ± 109.4**	**532.07 ± 54.36**	**631.89 ± 64.91**	**355.92 ± 10.53**	**655.21 ± 68.96**	**617.25 ± 28.75**	**701.38 ± 74.48**
33	trans-furan linalool oxide	16.07	90	5.45 ± 0.50	12.25 ± 1.53	13.38 ± 3.82	10.25 ± 0.66	12.16 ± 1.72	13.08 ± 2.50	7.72 ± 1.94	8.30 ± 1.28	16.21 ± 2.59	2.06 ± 0.10	nd	nd	nd
34	cis-furan linalool oxide	17.51	90	3.61 ± 0.67	6.64 ± 1.24	9.61 ± 2.21	10.14 ± 0.81	14.54 ± 2.97	13.52 ± 4.24	6.92 ± 1.28	6.71 ± 0.59	11.49 ± 3.81	6.32 ± 0.35	nd	nd	nd
35	linalool	21.91	98	nd	15.83 ± 4.88	7.65 ± 1.52	6.85 ± 2.16	nd	4.39 ± 0.96	9.88 ± 3.44	5.98 ± 1.35	9.94 ± 2.32	nd	nd	nd	nd
36	α-terpineol	29.65	98	nd	8.00 ± 2.49	9.97 ± 4.59	4.53 ± 0.39	8.65 ± 1.03	8.61 ± 1.28	5.31 ± 0.32	3.88 ± 0.40	7.21 ± 1.41	2.54 ± 0.28	3.61 ± 1.16	2.07 ± 0.10	nd
37	trans-pyran linalool oxide	31.93	75	6.24 ± 1.39	19.09 ± 2.45	24.05 ± 6.10	15.89 ± 2.46	23.20 ± 2.54	19.50 ± 3.24	14.05 ± 3.51	10.48 ± 1.34	21.76 ± 2.70	2.93 ± 0.62	nd	nd	nd
38	cis-pyran linalool oxide	33.44	75	2.23 ± 0.65	3.84 ± 0.42	5.45 ± 0.49	4.63 ± 0.78	10.38 ± 1.38	7.90 ± 2.49	4.12 ± 1.20	3.41 ± 0.48	5.82 ± 1.86	0.62 ± 0.14	nd	nd	nd
39	citronellol	33.85	80	2.85 ± 0.59	nd	nd	nd	nd	nd	nd	5.77 ± 1.72	nd	nd	3.13 ± 0.01	4.66 ± 0.01	nd
40	nerol	35.51	98	10.23 ± 1.23	19.27 ± 4.22	12.27 ± 3.93	9.36 ± 0.38	6.44 ± 1.64	10.32 ± 2.59	12.1 ± 2.86	12.46 ± 2.33	12.47 ± 3.09	5.61 ± 0.84	5.96 ± 1.13	5.85 ± 2.40	10.69 ± 3.05
41	geraniol	38.18	98	47.99 ± 0.66	35.40 ± 1.12	30.05 ± 6.68	24.56 ± 1.78	21.86 ± 2.30	22.53 ± 1.37	20.72 ± 4.31	29.27 ± 2.96	25.99 ± 4.73	11.49 ± 0.40	13.26 ± 2.21	6.71 ± 0.56	28.64 ± 1.75
42	diol (2,6-dimetil-3,7-ottadien-2,6-diolo)	43.52	95	3.48 ± 0.70	7.92 ± 1.81	8.80 ± 2.20	10.71 ± 0.23	6.79 ± 1.67	9.61 ± 2.20	9.6 ± 1.33	9.97 ± 0.80	6.67 ± 1.38	8.84 ± 0.17	nd	nd	nd
43	endiol	45.17	75	6.01 ± 0.57	nd	5.75 ± 2.04	4.87 ± 0.15	3.52 ± 0.52	nd	nd	5.48 ± 0.84	nd	2.20 ± 0.68	nd	nd	nd
44	cresol	46.04	90	nd	nd	nd	nd	2.66 ± 0.73	2.63 ± 0.65	nd	nd	nd	nd	nd	nd	4.14 ± 1.08
45	hydroxy citronellol	55.89	75	12.64 ± 2.93	nd	nd	nd	nd	nd	nd	nd	nd	5.94 ± 1.66	7.59 ± 0.41	8.06 ± 0.15	nd
46	8-hydroxydihydrolinalool	56.06	75	nd	8.97 ± 1.10	5.42 ± 0.52	7.25 ± 1.28	4.49 ± 0.96	5.91 ± 1.91	6.88 ± 0.91	11.40 ± 1.78	6.84 ± 1.72	3.65 ± 0.86	4.27 ± 0.52	5.41 ± 0.51	10.09 ± 2.46
47	trans-8-hydroxy-linalool	58.52	90	14.80 ± 3.25	38.50 ± 3.26	43.22 ± 7.45	43.42 ± 3.89	50.32 ± 9.13	34.41 ± 3.29	41.41 ± 4.23	38.55 ± 4.68	36.06 ± 3.31	23.49 ± 1.66	9.17 ± 1.31	7.55 ± 0.97	11.67 ± 1.13
48	hydroxy geraniol	59.94	75	30.34 ± 3.02	24.96 ± 3.86	16.52 ± 8.59	7.53 ± 0.94	5.57 ± 1.77	6.54 ± 0.70	12.21 ± 4.69	12.59 ± 3.06	8.66 ± 1.56	3.33 ± 0.19	4.38 ± 0.58	3.08 ± 0.31	12.25 ± 0.28
49	cis-8-hydroxy-linalool	59.97	75	39.96 ± 9.33	150.78 ± 10.54	102.16 ± 30.58	99.15 ± 5.19	60.72 ± 7.30	77.26 ± 11.40	107.68 ± 8.12	134.81 ± 9.41	124.74 ± 25.22	16.57 ± 0.57	22.76 ± 5.53	21.26 ± 6.81	68.41 ± 5.19
50	geranic acid	60.67	95	34.28 ± 1.76	71.47 ± 6.69	58.86 ± 9.82	55.39 ± 10.06	59.71 ± 4.64	47.51 ± 7.44	58.81 ± 8.12	80.35 ± 13.76	48.53 ± 8.93	32.20 ± 1.35	21.87 ± 2.78	22.71 ± 3.99	11.47 ± 1.11
51	p-menthene-7,8-diol	66.67	95	nd	12.23 ± 1.72	17.73 ± 7.32	9.52 ± 1.14	20.52 ± 8.59	17.01 ± 2.52	8.80 ± 2.02	12.11 ± 0.94	16.78 ± 4.47	5.85 ± 0.96	20.69 ± 6.79	15.20 ± 4.09	nd
	terpenes			**218.95 ± 17.90**	**435.13 ± 10.62**	**370.89 ± 90.70**	**324.04 ± 22.28**	**311.54 ± 30.43**	**300.72 ± 27.80**	**326.22 ± 20.15**	**391.52 ± 34.79**	**359.17 ± 38.01**	**133.64 ± 4.67**	**114.61 ± 10.86**	**99.45 ± 5.76**	**157.36 ± 8.28**
	Total			**1187.66 ± 110.49**	**1625.17 ± 108.85**	**1662.55 ± 245.96**	**1391.69 ± 92.45**	**1690.70 ± 123.8**	**1609.76 ± 59.89**	**1651.62 ± 239.48**	**1469.99 ± 33.70**	**1666.19 ± 142.24**	**1053.47 ± 8.59**	**1617.18 ± 93.3**	**1691.27 ± 127.71**	**2173.53 ± 84.86**

Table 3. Odour active value (OAV) and standard deviations in red varieties. Only compounds with OAV > 0.20 are shown (data are the average of the tree replicate samples; Keys in Chapter 6; Table 1). RT: Retention time; MQ (%): Match quality (%); nd: not detected

	Compounds	RT	MQ (%)	BAT.E23	BEN.E18	BOB.E11	BOB.E21	CAL.E26	EPE.E37	EXC.E27	FER.E22	FOG.E16	FOG.E24
5	benzaldehyde	20.11	95	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
8	benzyl alcohol	39.32	98	0.25 ± 0.05	0.83 ± 0.13	0.69 ± 0.06	0.67 ± 0.07	0.87 ± 0.18	0.44 ± 0.07	0.38 ± 0.06	0.68 ± 0.03	0.22 ± 0.05	0.19 ± 0.03
9	2-phenylethanol	41.03	98	0.12 ± 0.01	0.09 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	0.09 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.13 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
10	eugenol	53.55	98	1.87 ± 0.52	1.30 ± 0.29	1.49 ± 0.27	1.30 ± 0.18	nd	1.07 ± 0.05	1.02 ± 0.08	1.26 ± 0.12	nd	nd
11	4-vinylguaicol	54.88	95	0.24 ± 0.06	0.28 ± 0.08	0.34 ± 0.16	0.26 ± 0.08	0.29 ± 0.07	0.20 ± 0.10	0.35 ± 0.14	0.16 ± 0.00	0.28 ± 0.05	0.23 ± 0.02
12	4-vinylphenol	62.52	95	0.34 ± 0.16	0.14 ± 0.05	0.44 ± 0.12	0.19 ± 0.05	0.79 ± 0.01	0.31 ± 0.26	0.99 ± 0.54	0.12 ± 0.01	0.64 ± 0.04	0.80 ± 0.19
35	linalool	21.91	98	0.18 ± 0.00	nd	0.03 ± 0.01	0.03 ± 0.01	nd	nd	nd	nd	0.18 ± 0.06	nd
36	α-terpineol	29.65	98	0.14 ± 0.03	0.13 ± 0.02	nd	nd	0.08 ± 0.02	nd	0.05 ± 0.01	0.02 ± 0.00	0.05 ± 0.01	0.08 ± 0.01
39	citronellol	33.85	80	nd	nd	0.07 ± 0.01	0.10 ± 0.02	0.18 ± 0.01	0.18 ± 0.04	0.18 ± 0.03	0.34 ± 0.12	0.27 ± 0.11	0.11 ± 0.00
40	nerol	35.51	98	0.35 ± 0.05	0.07 ± 0.02	0.11 ± 0.04	0.12 ± 0.01	0.11 ± 0.04	0.11 ± 0.02	0.07 ± 0.01	0.32 ± 0.02	0.07 ± 0.03	0.04 ± 0.00
41	geraniol	38.18	98	2.39 ± 0.27	0.58 ± 0.15	0.78 ± 0.11	0.75 ± 0.08	0.63 ± 0.05	0.56 ± 0.14	0.42 ± 0.11	1.25 ± 0.13	0.54 ± 0.03	0.45 ± 0.08

	Compounds	RT	MQ (%)	GAL.E15	GIR.E31	GIR.E36	GOR.E25	MES.E30	MTN.E20	MTN.E29	PAM.H24	SAB.E35
5	benzaldehyde	20.11	95	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
8	benzyl alcohol	39.32	98	0.31 ± 0.04	0.20 ± 0.04	0.20 ± 0.02	0.26 ± 0.04	0.35 ± 0.03	0.50 ± 0.13	0.50 ± 0.16	0.38 ± 0.05	1.18 ± 0.10
9	2-phenylethanol	41.03	98	0.04 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.06 ± 0.00	0.10 ± 0.00	0.10 ± 0.03	0.10 ± 0.04	0.09 ± 0.00	0.14 ± 0.01
10	eugenol	53.55	98	1.18 ± 0.17	nd	nd	1.85 ± 0.53	nd	nd	nd	nd	1.28 ± 0.31
11	4-vinylguaicol	54.88	95	0.23 ± 0.05	0.22 ± 0.08	0.13 ± 0.01	0.35 ± 0.06	0.28 ± 0.05	0.25 ± 0.07	0.23 ± 0.07	0.18 ± 0.02	0.41 ± 0.09
12	4-vinylphenol	62.52	95	0.55 ± 0.16	0.27 ± 0.06	0.27 ± 0.05	0.22 ± 0.11	0.35 ± 0.21	0.22 ± 0.14	0.20 ± 0.07	0.28 ± 0.07	0.30 ± 0.07
35	linalool	21.91	98	nd	1.53 ± 0.33	3.74 ± 0.73	nd	nd	0.25 ± 0.00	0.24 ± 0.00	0.37 ± 0.08	nd
36	α-terpineol	29.65	98	0.02 ± 0.01	0.24 ± 0.02	0.31 ± 0.05	0.34 ± 0.04	0.14 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.21 ± 0.03	nd
39	citronellol	33.85	80	nd	0.21 ± 0.03	0.11 ± 0.00	nd	nd	nd	nd	nd	nd
40	nerol	35.51	98	0.07 ± 0.01	0.09 ± 0.03	0.10 ± 0.01	0.07 ± 0.01	0.13 ± 0.04	0.09 ± 0.03	0.10 ± 0.03	0.16 ± 0.04	0.06 ± 0.00
41	geraniol	38.18	98	0.23 ± 0.07	1.07 ± 0.10	1.69 ± 0.21	0.39 ± 0.10	1.57 ± 0.20	0.55 ± 0.15	0.53 ± 0.05	2.04 ± 0.23	0.37 ± 0.01

Table 4. Odour active value (OAV) in white and rose varieties and standard deviations. Only OAV > 0.20 are shown (data are the average of the tree replicate samples; Keys in Chapter 6; Table 1). RT: Retention time; MQ (%): Match quality (%); nd: not detected

	Compounds	RT	MQ (%)	ARG.E34	BEB.E14	BEB.E32	BEB.E39	BEB.E43	BEB.O16	BEB.O17	BEB.O44	BER.E19
5	benzaldehyde	20.11	95	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	nd
8	benzyl alcohol	39.32	98	0.31 ± 0.01	0.33 ± 0.05	0.49 ± 0.12	0.35 ± 0.03	0.19 ± 0.02	0.45 ± 0.04	0.43 ± 0.04	0.29 ± 0.04	0.34 ± 0.11
9	2-phenylethanol	41.03	98	0.13 ± 0.01	0.16 ± 0.02	0.19 ± 0.04	0.15 ± 0.01	0.19 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.14 ± 0.01	0.20 ± 0.04
10	eugenol	53.55	98	1.14 ± 0.25	0.61 ± 0.11	1.30 ± 0.35	1.06 ± 0.35	0.53 ± 0.17	1.07 ± 0.21	1.43 ± 0.50	0.80 ± 0.19	1.14 ± 0.37
11	4-vinylguaicol	54.88	95	0.26 ± 0.03	0.25 ± 0.06	0.20 ± 0.02	0.24 ± 0.01	0.23 ± 0.04	0.29 ± 0.06	0.34 ± 0.11	0.33 ± 0.06	0.28 ± 0.01
12	4-vinylphenol	62.52	95	0.08 ± 0.01	0.10 ± 0.03	0.05 ± 0.01	0.04 ± 0.00	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.11 ± 0.05	0.06 ± 0.01
35	linalool	21.91	98	nd	1.02 ± 0.33	0.52 ± 0.11	0.47 ± 0.15	nd	0.29 ± 0.07	0.72 ± 0.25	0.46 ± 0.11	0.74 ± 0.19
36	α-terpineol	29.65	98	nd	0.10 ± 0.03	0.12 ± 0.06	0.06 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.07 ± 0.00	0.06 ± 0.01	0.10 ± 0.02
39	citronellol	33.85	80	0.17 ± 0.03	nd	nd	nd	nd	nd	nd	0.37 ± 0.11	nd
40	nerol	35.51	98	0.14 ± 0.03	0.23 ± 0.05	0.16 ± 0.07	0.12 ± 0.00	0.08 ± 0.02	0.13 ± 0.03	0.17 ± 0.04	0.18 ± 0.04	0.17 ± 0.04
41	geraniol	38.18	98	1.70 ± 0.18	1.14 ± 0.05	1.04 ± 0.33	0.85 ± 0.07	0.70 ± 0.09	0.74 ± 0.04	0.76 ± 0.17	1.12 ± 0.13	0.96 ± 0.18

	Compounds	RT	MQ (%)	PEN.SC	QUI.E33	QUI.E38	VIN.E45
5	benzaldehyde	20.11	95	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.29 ± 0.01
8	benzyl alcohol	39.32	98	0.28 ± 0.01	0.39 ± 0.06	0.50 ± 0.09	0.54 ± 0.02
9	2-phenylethanol	41.03	98	0.08 ± 0.00	0.14 ± 0.01	0.13 ± 0.00	0.13 ± 0.01
10	eugenol	53.55	98	0.77 ± 0.02	1.00 ± 0.23	0.86 ± 0.18	2.01 ± 0.34
11	4-vinylguaicol	54.88	95	0.43 ± 0.02	0.48 ± 0.12	0.42 ± 0.06	0.36 ± 0.05
12	4-vinylphenol	62.52	95	0.03 ± 0.01	0.10 ± 0.02	0.12 ± 0.02	0.06 ± 0.01
35	linalool	21.91	98	nd	nd	nd	nd
36	α-terpineol	29.65	98	0.03 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	nd
39	citronellol	33.85	80	nd	0.16 ± 0.00	0.20 ± 0.00	nd
40	nerol	35.51	98	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.02	0.08 ± 0.02
41	geraniol	38.18	98	0.40 ± 0.01	0.36 ± 0.05	0.16 ± 0.01	0.60 ± 0.03

Figure 1. Typical chromatogram of a red variety

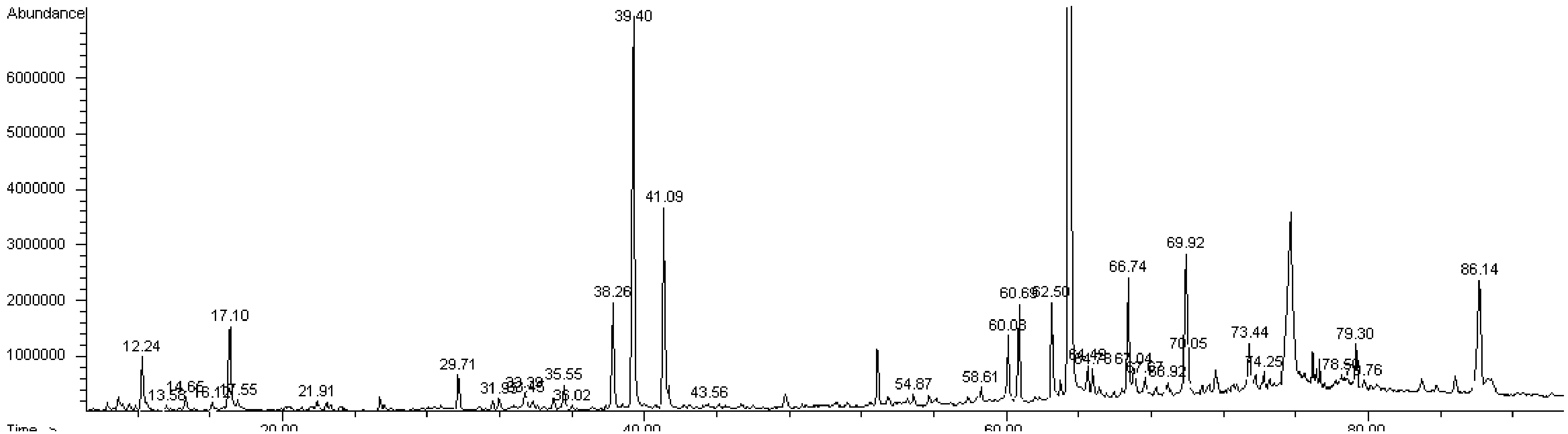
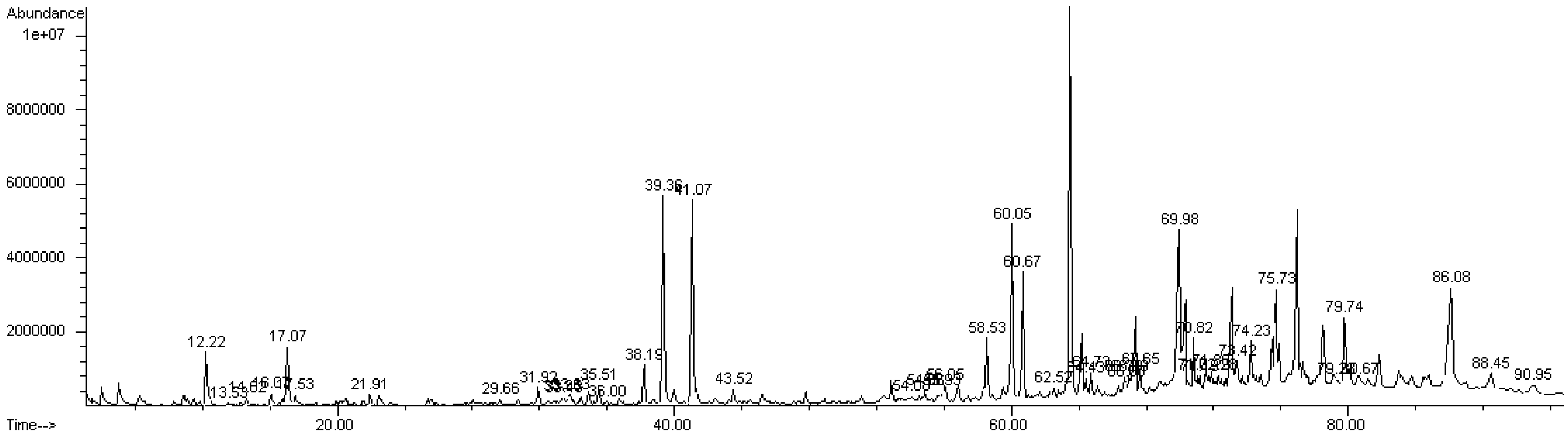


Figure 2. Typical chromatogram of a white variety



Appendix IV: Resumen en castellano

Resumen Capítulo 2: Evidencias de la pérdida de diversidad de vides cultivadas: el ejemplo de las Islas Baleares (España)

La pérdida de variedades de vid en todo el mundo ha producido una importante erosión genética del material de base. Este problema es más notable en áreas aisladas por su particular situación, caracterizada por especímenes únicos, como es el caso de las Islas Baleares (España).

El objetivo de este trabajo fue cuantificar la pérdida de variedades de vid desde el siglo XVII hasta la actualidad, y para alcanzarlo, fue necesaria una minuciosa investigación para poder identificar los nombres de las variedades de vid encontrados en la bibliografía antigua. Con el fin de comparar las descripciones ampelográficas antiguas con las variedades conservadas en la actualidad, se utilizaron las descripciones ampelográficas encontradas en la literatura consultada, así como la información disponible en dos bancos de germoplasma. En este trabajo se discuten las posibles causas que han cambiado la viticultura balear, tales como diferentes enfermedades (oídio, pulgón o la plaga filoxérica), la influencia de las Denominaciones de Origen sobre la evolución del número de cultivares y su superficie de cultivo o la entrada de variedades foráneas.

Más del 75% de las variedades encontradas en la bibliografía antigua pudieron ser identificadas. Uno de los resultados más interesantes fue la gran diversidad de variedades de vid encontradas en las Islas Baleares a pesar de poseer un área geográfica reducida, lo que convierte a estas islas en un importante reducto para diferentes variedades. Así mismo, y contrariamente a lo que se pensaba, el mayor cambio que ha sufrido la viticultura balear se ha datado antes de que la plaga filoxérica entrara en las islas. Las islas fueron divididas en diferentes áreas de viticultura, cada una de ellas con una variedad de referencia. La metodología utilizada en este trabajo ha resultado útil para cuantificar la pérdida de diversidad genética en vid. Este trabajo ha permitido concluir que, desafortunadamente, algunos de los antiguos cultivares se han se han perdido o se encuentran en peligro de extinción en la actualidad, habiéndose cuantificado una importante pérdida de diversidad genética, alrededor del 50%, a lo largo del periodo estudiado.

Palabras clave: Conservación genética, evolución del cultivo, variedades minoritarias, *Vitis vinifera*

Resumen Capítulo 3: Ampelografía: una vieja técnica con usos futuros, el caso de variedades minoritarias de vid (*Vitis vinifera* L.) de las Islas Baleares

El objetivo de este trabajo fue evaluar las descripciones ampelográficas, análisis genético, caracterización agronómica, variables de mosto y fenología de 27 accesiones minoritarias de vid procedentes de las Islas Baleares (España). También se ha estudiado la influencia de un fenómeno climático ocasional (granizada) sobre las variables agronómicas estudiadas y sobre las descripciones ampelográficas realizadas. Así mismo, se ha evaluado la influencia de la experiencia de los ampelógrafos sobre las descripciones ampelográficas. Las accesiones de vid fueron analizadas utilizando 58 descriptores OIV, tanto cualitativos como cuantitativos, y seis marcadores moleculares (nuSSR).

Nuestros resultados muestran que la ampelografía es una buena técnica preliminar para clarificar la identidad del material vegetal, lo cual se ha confirmando también mediante marcadores moleculares. El color de la hoja joven (OIV-051), la jugosidad de la pulpa (OIV-232) y la firmeza de la pulpa (OIV-235) han sido los caracteres más difíciles de distinguir por los ampelógrafos. A pesar de la gran similitud encontrada entre las variedades estudiadas, hubo ciertos caracteres clave para la identificación de estas variedades (OIV-225, OIV-084, OIV-053, OIV-004). Además, un fenómeno climático puntual (granizada) influenció sobre las descripciones ampelográficas, los parámetros agronómicos y la fenología.

La caracterización morfológica y molecular de las 27 accesiones de vid recolectadas en las Islas Baleares ha permitido validar el método de descripciones ampelográficas agrupándolas en 17 variedades de vid diferentes. El análisis genético ha mostrado que Beba blanca puede ser una posible mutación somática de Beba roja. Finalmente, se ha observado que la granizada incrementó el periodo vegetativo de las vides, afectando especialmente a la hoja madura, al racimo, a las características agronómicas y a la composición del mosto.

En este trabajo se han caracterizado por primera vez los perfiles ampelográficos y moleculares de estas variedades de vid minoritarias, mostrando el potencial y el interés de éstas y sugiriendo que su utilización podría ser importante para los viticultores.

Palabras clave: Morfología, variedad de vid, caracterización agronómica, descriptores, influencia de la granizada

Resumen Capítulo 4: Variedades de vid (*Vitis vinifera* L.) de las Islas Baleares: caracterización genética y relaciones con la Península Ibérica y la cuenca Mediterránea

Se ha realizado la identificación de 66 accesiones de *Vitis vinifera* L. a través de descripciones ampelográficas, un grupo de 20 marcadores microsatélites nucleares (nuSSR), cinco microsatélites cloroplásticos (cSSR), así como mediante referencias históricas. El material vegetal incluye vides mayoritarias y minoritarias en riesgo de extinción recolectadas en las Islas Baleares y ahora conservadas en dos bancos de germoplasma españoles.

Los resultados de este trabajo señalan que las 66 muestras analizadas se corresponden con 32 genotipos diferentes, entre los que se encuentran algunos genotipos únicos, tres de los cuales son desconocidos. Se han descubierto diferentes sinonimias y homonimias en la cuenca Mediterránea, indicando que la dispersión de algunas variedades está relacionada con movimientos humanos históricos y migraciones ocurridas en tres periodos, (1) alrededor del siglo VII relacionado con la expansión del Islam, (2) entre los siglos XIII-XV y (3) en el siglo XIX relacionado con la crisis filoxérica.

Se han identificado algunas relaciones de parentesco entre las variedades estudiadas, siendo Callet Cas Concos una variedad clave en muchos cruzamientos, lo que confirma el alto valor que presentan las variedades desconocidas en los análisis de parentesco. Los diferentes métodos de agrupación utilizados han confirmado la existencia de dos reservas genéticas.

Palabras clave: microsatélites nucleares, clorotipo, variedades minoritarias, estructura genética, análisis de parentesco

Resumen Capítulo 5: Evaluación de la susceptibilidad al oídio (*Erysiphe necator*) en variedades de *Vitis vinifera*

Se ha evaluado la susceptibilidad al oídio (*Erysiphe necator* Schwein.) en 159 variedades de vid tanto extranjeras como autóctonas que se cultivan en España. También se ha estudiado la relación entre caracteres morfológicos y su susceptibilidad a la enfermedad. La infección de las variedades de vid se ha estudiado en condiciones naturales tanto en hoja como en racimos. Se ha encontrado que 35 variedades fueron muy susceptibles a la enfermedad (de muy baja a baja resistencia en racimos), mientras que otras 83 variedades mostraron baja susceptibilidad (de alta a muy alta resistencia en racimos). Los resultados proporcionan una información útil en la selección de variedades menos susceptibles al oídio tanto para viticultores como para mejoradores.

Palabras clave: *Erysiphe necator*, morfología, oídio, susceptibilidad, *Vitis vinifera*

Resumen Capítulo 6: Caracterización aromática y potencial enológico de 21 variedades minoritarias de vid (*Vitis vinifera* L.)

La homogenización del mercado internacional del vino está dando lugar a un gradual empobrecimiento de la reserva genética de los viñedos. De hecho, en diferentes Denominaciones de Origen españolas las variedades de vid internacionales están frecuentemente reemplazando a las variedades locales, lo que provoca que las variedades minoritarias, perfectamente adaptadas a las condiciones medioambientales, estén en la actualidad en riesgo de extinción. El estudio de variedades minoritarias podría proporcionar información útil para satisfacer la demanda de nuevos productos vitícolas. Este trabajo pretende cubrir la falta de información relativa al potencial aromático de variedades minoritarias, por lo que se ha considerado el estudio de compuestos volátiles glicosilados y la evaluación de la influencia de diferentes variables sobre la composición aromática de las uvas. Se han identificado y cuantificado 51 compuestos glicosilados. Para poder identificar los compuestos aromáticos más potentes de las variedades estudiadas se han utilizado "Odour Activity Values" (OAVs). En la evaluación del OAV, las series odorantes más importantes fueron floral, especiada y fenólica. Los resultados revelan diferencias para los compuestos glicosilados teniendo en cuenta la variedad, el color de la baya, el clon y el origen de las muestras. Por otra parte, la síntesis de algunos compuestos implicados en estas diferenciaciones parece tener un componente genético. La caracterización del potencial aromático de diferentes variedades minoritarias de vid, llevadas a cavo por primera vez, revelan que algunas de ellas (Argamusa, Gorgollassa and Pampolat girat) podrían representar una excelente opción para los enólogos y para desarrollar estrategias de diversificación comercial, además de ser importante para la conservación de estas variedades.

Palabras clave: Aroma, glicósidos, cromatografía de gases, "flavor"

Resumen Capítulo 7: Caracterización sensorial y factores que influyen en la calidad de los vinos elaborados a partir de 18 variedades minoritarias de vid (*Vitis vinifera* L.)

El mercado enológico está vivo, siempre en busca de nuevas variedades para satisfacer la demanda de los consumidores. De este modo, las variedades minoritarias podrían ser firmes candidatas para satisfacer esta demanda emergente, sin embargo, no se conoce el potencial enológico de la mayoría de estas variedades. Aunque la calidad de los vinos es difícil de evaluar, los análisis cuantitativos y descriptivos son los métodos más utilizados para la caracterización sensorial de los vinos. El objetivo de este trabajo fue caracterizar vinos elaborados con variedades locales utilizando la descripción sensorial. Se analizaron sensorialmente en dos vendimias vinos elaborados a partir de 18 variedades minoritarias de vid, incluyendo vinos tintos, blancos y rosados. El análisis de la influencia de la vendimia, de la inclusión de la variedad en Denominación de Origen y de los parámetros agronómicos sobre los atributos sensoriales evaluados por 21 expertos catadores de vino, ha sido realizado mediante análisis de la varianza, análisis de componentes principales y "partial least squares". Se ha realizado además un mapa de preferencia de los catadores para identificar las posibilidades de estos vinos.

Este trabajo destaca que el cuestionario utilizado y los expertos fueron eficientes para evaluar los vinos catados y que el efecto vendimia ha sido más importante que el efecto Denominación de Origen sobre la caracterización química y sensorial de los vinos. El análisis sensorial realizado sobre estos vinos ha demostrado una correlación significativa entre los atributos sensoriales y los parámetros agronómicos evaluados. Los parámetros agronómicos vegetativos y productivos tienen una influencia inversa sobre los parámetros aromáticos y el sabor de los vinos dado por los expertos, por lo que el manejo de estos parámetros en campo podría mejorar la calidad de los vinos. Este estudio también muestra las posibilidades que podrían tener en el mercado enológico las variedades estudiadas, especialmente aquellas que han sido aceptadas por los expertos catadores. Las variedades localizadas en la mejor posición del mapa de preferencia de los catadores deberían ser consideradas por los técnicos debido a su calidad. Algunas de estas variedades minoritarias han sido incluso mejor posicionadas que los vinos elaborados con variedades permitidas en Denominaciones de Origen españolas. Esta información podría despertar un mayor interés sobre variedades minoritarias o autóctonas, siendo clave para su conservación.

Palabras clave: preferencia de los expertos, variedades locales, análisis sensorial, vendimia, calidad del vino