PRIORAT VINEYARD VULNERABILITY AND WATER STRESS ASSESSMENT IN THE CONTEXT OF GLOBAL CLIMATE CHANGE. ESTIMATED PRIORAT WINE CONSUMPTION IN HUMANS

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DOCTORAL THESIS

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Tarragona
2021

UNIVERSITAT ROVIRA I VIRGILI
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Preface

Preliminary studies on the behaviour of *Vitis vinifera* Grenache and Carignan vines has enabled the VTV Viticulture Research group at the University Rovira i Virgili to achieve a high level of understanding of the physiology of this cultivar in areas of a warm Mediterranean climate. The initial project I was involved with was the wine-growing region of Terra Alta (Tarragona, Spain) which allowed me to understand the behaviour of the physiology of the vines during the vegetative growth cycle and fruit ripening. I joined in the study of varieties Grenache and Carignan. The first showed a very different bunch ripening behaviour related to the mesoclimate. Grenache and Carignan are the more abundant varieties of the Priorat wine region where I spent the last twenty years making wine so I was interested to do a deeper study.

The appellation is known worldwide for the excellent quality of its wines and had great impact on the economic development of the region in recent years. Monitoring the behaviour of the two varieties is of great importance in the future of the region. Deeper understanding of the ripening of fruit at harvest and various physiological responses and the level of isohydry of Grenache and Carignan lead us to study the Priorat as a very vulnerable Mediterranean wine-growing region in the context of global climate change. In addition, it is crucial to see whether this vulnerability is sustainable in terms of production and quality of the resulting wines. For this reason the thesis is divided into three main chapters: 4, 5 and 6.

Chapter 4 is focused on the characterisation of cv. Grenache and Carignan, mesoclimate and soil, vegetative growth and physiological parameters in order to assess plant stress environment. This will establish two main locations, early and a late ripening mesoclimates. Parameters of climate and soil will be deeply analysed to determine how the vegetative development of the vine and in particular how water stress affect the synthesis of plant hormones; mainly abscisic acid which is a good indicator of the level of water stress in vines. A simplified method for the determination of this phytohormone in leaf vines has been developed, together with the growth measurements. This section will go further deep into the case of Carignan and Grenache varieties at the level of secondary metabolism in grapes, to deepen in the phenolic compounds that accumulate in fruit and which factors determine their quality into the wine.

Chapter 5 escribes and analyses a methodology of small-scale winemaking based on notable heterogeneity found in previous research. Small-scale fermentation protocols used mirrored typical winemaking techniques commonly used in the small production wineries of the Priorat. By applying this methodology to Grenache and Carignan, grapes

were processed and turned into wine for analysis. The phenolic composition of the wine has been essential to establishing quality parameters and to assess the consequence of the water stress during crucial periods.

Chapter 6 evaluates the potential consumption of Priorat wine based on a bibliographic research I did for my *Master of nutrition and metabolism*. The numerous studies that refer to the effect of wine consumption on health show a lack of detail on the quality of the wines consumed, both in their initial composition and in their geographical origin. Given the interest of many organizations in guaranteeing the origin of wine from a certain geographical area, analysis of the impact of the consumption of a specific region, in this case Priorat was evaluated. Another investigation in this section assesses how wine, in moderate consumption can reduce caloric intake but without losing its potential antioxidant capacity and beneficial health effects.

The analysis of soils and phenolic maturity were carried out in the laboratories of the Department of Biochemistry and Biotechnology of the Faculty of Enology and the grape samples in the laboratory of *Mas dels Frares* in Constantí. The acid abscisic (ABA) and HPLC analyses were carried out at the headquarters of Shirota Functional Foods, SFF in Reus. Plant measurements were carried out in the field directly with mobile equipment. All experiments were carried out under field conditions.

Dedication

To my grandmother
who taught me to value life
respect others
work hard
and above all
believe in possibility

Acknowledgments

This thesis, being the shortest, ends up being the longest in history. Not only because of the stages it has gone through but because of the people involved along the way. It began as a study on traditional varieties in Terra Alta and Priorat, then it became part of a larger project related to Climate Change. Finally it was put aside for a few years due to issues that would require another thesis; and finally during the Covid-19 it ends up being re-organized and making it reality. Things happen when they are ready, and in this case it came in a pandemic! Why not.

I appreciate the help of several people, each of whom have added knowledge, experiences, adventures, friendship, frustration and every other possible emotion. So, first of all, I would like to thank the director of this thesis, Josep Maria Mateo-Sanz, PhD, whose patience and perseverance have enabled me to resume this thesis with enthusiasm, encouraging me to recover previous data and giving them relevance.

I would like to acknowledge the University Rovira i Virgili, Biochemistry Department, VTV Viticulture Research Group. To the professors of the Faculty of Enology, Montse Nadal, PhD, who introduced me to the world of research and who guided me for a long period of time; Miriam Lampreave, PhD, who gave me support and helped during the measurements and and Nicolàs Rozés, PhD, who helped with data analysis.

Secondly I would like to thank Josep Valls, PhD, who helped me proceed with all the chromatographic analyses of the Priorat wines; Silvia Millán, PhD, and Maria Pilar Martí, PhD, who helped a lot during the development of a methodology for the extraction of phytohormones from Carignan leafs. And in general to all the scholarship students who so generously supported the work with their time. I am grateful for the good moments I shared with them. I give special thanks to Marina Borràs for her perseverance, Tomàs Viñas for his great spirit while working so late into the night finishing most of the analysis. To Coline Leriche (currently a PhD), Vanessa Françon (great student and better person), Loïc Aznar (amazing surfer), Maria Azuara (The Mexican wine lover), David Barriche (Oenologist from the URV), Joan Carles Nadal (Chemist from the URV), Daniel Sánchez (Biochemist from the URV), Enric Juan Juvanteny (Oenologist from the URV) and Franco Vittorini (Chemist and friend). Whatever they did, this is just an example.

Particularly, I am very grateful for the period when I was working for the IRTA, Institut de Recerca i Tecnologia Alimentàries, in the plant ecophysiology research group in Torre Marimon, Caldes de Montbui, where I learned everything I know about vine physiology and how to understand water stress. I extend my gratitude to Felicidad de Herralde, PhD,

Robert Savé, PhD, Carme Biel, PhD, Gonzaga Santesteban, PhD (Currently a professor at the UPNa) and Xavi Aranda, PhD, and Beatriz Grau, a scholarship and friend.

Thanks to the owner of the El Molar vineyards, Josep Anguera, for helping me when needed and for his predisposition to the *Mas dels Frares* vineyard support, as well as Josep Maria Alsina and Pedro Cabanillas, for giving me access to the facility when working extra hours, before sunrise and after sunset, measuring water stress parameters. Also to Isabel Fortuny, for letting us carry out measurements on the Porrera vineyards, managed by Ferrer Bobet.

A special dedication to Cinta Bladé (R.I.P), PhD, who introduced me to the world of Human Nutrition and Metabolism during the MS and who supported my bibliographic review on *Estimated Priorat Wine Consumption In Humans* in Chapter 6.

Partial support for this work has been received from the Spanish Government (project RTI2018-095094-B-C21 "CONSENT") and by a National Cicyt project (AGL 2008-04525-CO2-O2). This thesis was also partially funded by the URV within the project 2009/BRDI/06/20 at the Biochemistry and Biotechnology department.

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Abbreviations

ABA ..... Abscisic Acid
ABV..... Alcohol by volume
AFT ..... Area Foliar Total
AG ..... Acetyl glucosides
ANOVA ..... Analysis of the variance
CART ..... Correlation and regression trees
CG ..... Coumaroyl-glucoside
CS ..... Cabernet Sauvignon (Grapevine)
CV..... Coefficients of variation
DAD..... Diode Array Detector
DMAC ..... *p*-dimethylaminocinnamaldehyde
DOGC ..... Diari Oficial de la Generalitat de Catalunya
AOC ..... Appellation d'Origine Controlée
DO ..... Denominació d'Origen
DOQ..... Denominació d'Origen Qualificada
EM ..... Acronym for El Molar
EMBA ..... Acronym for El Molar Vineyard, located down the hill, Site 2
EMDA..... Acronym for El Molar Vineyard, located up the hill, Site 1
EMGRE..... Acronym for El Molar Vineyard, located in terraces, Site 6
ESI-MS..... Electrospray ionization-Mass Spectrometry
ET_0 ..... Evapotranspiration
GC ..... coumaroyl glucoside
GDD_{10}..... Growing Degree Days
g_s..... Stomatal conductance
HPLC ..... High performance liquid chromatography
Kc..... Crop Coefficient
Kh ..... Hydraulic conductivity
LLOGRE..... Acronym for El Lloar Vineyard, located in terraces, Site 7
LO..... Acronym for El Lloar Vineyard, located in terraces, Site 6
LWP ..... Leaf Water Potential
MS..... Mass spectrometry
NDVI..... Normalised Difference Vegetation Index
NMR..... Nuclear Magnetic Ressonance
OIV..... Office International de la vigne et du vin
P..... Rainfall
PAR..... Photosynthetically Active Radiation
PC ..... Principal component
PCA ..... Principal Component Analysis
PH ..... Phenological stage of Post-Harvest
PLWP ..... Predawn Leaf Water Potential
PO..... Acronym for Porrera
PODA ..... Acronym for Porrera Vineyard, up the hill, located mid-west, Site 5

POME ..... Acronym for Porrera Vineyard, down the hill, located mid-east, Site 4POME..
POMO ..... Acronym for Porrera Vineyard, down the hill, located mid-west, Site 3
PS..... Phenological stage of Pea Size
R-110 ..... Richter-110 (Rootstock)
RP..... Phenological stage of Ripeness
RRLC..... Rapid Resolution Liquid Chromatography
TE..... Tempranillo (Grapevine)
TLA ..... Total Leaf Area
TOF..... Time of flight
TOFMS ..... Time of Flight Mass Spectrometer
Ts ..... Surface Leaf Temperature
TTA ..... Total titratable acidity
USDA ..... United States Department of Agriculture
UTM..... Universal Transversal Mercator
V..... Phenological stage of Veraison
VQPRD ..... Wines of Quality Produced in Determined Regions
VPD..... Vapor Pressure Deficit
VSP ..... Vertical Shoot Positioning
VWC ..... Volumetric water content

Chapter 1. Introduction

In the late 19th century the phylloxera epidemic destroyed most of the vineyards in Europe, which resulted, between 1874 and 1903, 30% loss for the surfaces of vines. Despite its consequences on the landscape, the Priorat has still retained traces that constitute assets of the surviving vineyards: walls, stone walling, terraces, villages, wine abbeys like Scala Dei, olive oil mills, ancient machinery museums, etc. Today, more than the great difficulty of cultivating the vineyards, the higher risk of abandonment is great. The defence of landscapes, it takes money and professionals, due to the difficulty of dealing with viticulture on high steep slopes. Nonetheless, the wine landscape is an important component of the wine origin and it summarizes the climate and soil for grape quality, the local history and the grape production traditions. Some experimental results reinforce that the conservation and valorisation of the mountain landscape, related with the vine potential of the mountain environment, is an important goal to achieve.

Landscapes are representative of the different wine regions of the world. Nature and humankind, they express a very close relationship between peoples, with powerful beliefs and traditional customs, together with their natural environment. Tarragona Wine County, being Tarraco the capital of Tarraconensis, the name given by Augustus to Hispania Citerior, already featured in the writings of the classical authors, such as Pliny the Elder and Silius Italicus. Undoubtedly, Romans possessed the predatory instinct that led them to build and empire and exploit the natural sources of their provinces. Grapevines were planted throughout the empire wherever the conditions were suitable, including such a poor and rocky soils, like those of the Priorat. There is absolutely no doubt about the historical origin of the name Priorat, since the region comprised the domains of the prior of the Carthusian monastery of Scala Dei. Nowadays, the Priorat DOQ reflects not only the history, landscape, flora, fauna and geology, but also the surprising black slate slopes where weak old vines grow. Consequently, its landscape properly represents the inland Mediterranean world: cultivated terraces on lofty mountains, forests, sacred places testifying the creative genius, social development and the imaginative and spiritual vitality of humanity. To reveal and sustain the great diversity of the interactions between humans and their environment, to protect living traditional cultures and preserve the traces of those which have disappeared, these cultural landscapes, must be defended. Priorat heritage allows the authenticity of its wine to survive and make people community to be economically sustainable.

The wine-growing area classified as Denominació d'Origen Qualificada Priorat (DOQ) is located in the central part of the province of Tarragona, in a depression caused by the doubling of the Serra del Montsant in its southern part. It is made up of land located in the municipalities of La Morera del Montsant - and its aggregate Scala Dei -, La Vilella Alta, La

Vilella Baixa, Gratallops, Bellmunt del Priorat, Porrera, Poboleda, Torroja del Priorat, El Lloar and the northern part from the municipalities of Falset and El Molar. Vine cultivation is distributed at altitudes ranging from 100 m above sea level, in part of the municipalities of Bellmunt del Priorat and El Molar, and up to 700 m, in the municipality of La Morera del Montsant. The crop configuration is characterized by slopes that exceed 15% in most cases to a maximum of 60%. Lately, terraces or terraced fields are being replanted that allow the mechanization of cultivation. It is one of the oldest Denominations of Origin in Spain, renowned and known for the production of reds with body and high alcohol content. The typical soil formed by schists allows rapid drainage that together with the hot summers and low rainfall characteristic of the region, configures an ecosystem in which drought prevails. The progressive depopulation of the area meant a reduction of the vineyard to an area of about 700 hectares at the end of the 80s. The decade of the 90s underwent a strong reconversion, passing in a short time from the sales of wine in bulk (half a million litres) to the sales of almost all bottled production. The wines are sold at a very high price and are highly valued for export. Today, the vineyard area is estimated at 2.088 hectares in 2020 and grape production averaged 6.3 million kg between 2015 and 2018 (www.doqpriorat.org). Strong climatic events have decreased the production to 5.5 in 2019 and to 4.1 in 2020. These unexpected weather conditions make this area a case study of high interest not only at a physiological level but also at an agricultural and socioeconomic level.

The last three decades, terracing of hillsides of Priorat has been extended to adapt the vineyard work to mechanization. However, some studies carried out to evaluate this impact of terracing on the vineyards of Priorat DOQ would indicate that, not only alters the morphology of the landscape but in addition it can be affected the production and quality of grapes. Soil hydrological properties are markedly affected, with a negative repercussion on water availability for plants, a reduction of soil water capacity and hydraulic conductivity of vines. In addition, terraces are constructed with higher risers and widths than those expected for the high slopes degrees existing in the area, with the result of landslides after not very few rainfall events. The maintenance or restoration of the risers is not carried out due to difficulties for heavy machinery accessibility and to avoid further damage to infrastructures such as irrigation, training systems and vines, damaged by mass movements. Hence, it was never of such importance to protect the Priorat landscape, to avoid mistakes of the past and preserving nowadays the beauty of its richness. As Carthusians did in the Priorat at the monastery of *Scalada dei*, these sorts of landscapes are an element of the multifunctionality of viticulture, and are vectors of historical, cultural and environmental communication, enriching of our wine-producing regions.

Grape composition and the type and style of wine in a given region are the result of the interaction of the combination of climate-soil with human activity, while the interannual

climate variability makes the difference in quality between vintages. Following climate warming predictions on a more global scale, increasing temperatures and decreasing precipitation together with the irregularity of their distribution, will more negatively affect Mediterranean agriculture. The grapevine is known as a type of crop well-adapted to water stress conditions; an example of this is the vineyards of the Priorat, characterized by low vigour plants growing on steep slopes and poor soils. However, the Priorat vineyard ecosystems could easily become vulnerable to the more severe weather conditions anticipated in the future. The concept of 'terroir' has a clear importance from an agricultural point of view. This concept refers initially to soil factor, includes pedology, climate and topography factors, interacting together in a particular agricultural unit under human influence. The integrated soil environment within a context of mesoclimate will have a different impact in the same crop in terms of characteristics presented by the system. A vineyard reflects its immediate growing area, including the soils and climatic conditions that influence production.

Variations resulting from the current climate change, especially in regions like the Mediterranean basin, should be carefully analysed and characterized for greater understanding. From the last report of the Mediterranean Experts on Climate and Environmental Change (MedECC, 2019), average annual air temperatures are now approximately 1.5°C higher than during the preindustrial period, well above current global warming trends (+1.1°C). Without additional mitigation, regional temperature increase will be of 2.2°C in 2040, possibly exceeding 3.8°C in some regions in 2100. Summer rainfall will decrease by 10 to 30% according to the area. Extreme events (heat waves, droughts, floods and fires) become more frequent. Surface seawater temperature has recently increased by about 0.4°C per decade. The projections for 2100 vary between +1.8°C and +3.5°C in average compared to the period between 1961 and 1990. Such climatic changes quickly effect growing regions featuring poor, coarse-textured soils with low fertility, especially those located in areas with low and irregular precipitation, and also subjected to erosive phenomena. Water stress, resulting from high evapotranspiration, lack of summer rainfall, and well-drained soils with low retention capacity, has a significant effect on such vineyards. An understanding of vegetative growth, and how this affects the final composition of the grapes, is a formula essential to determining optimal harvest dates for high quality wines. This study evaluates the effect of mesoclimate variability in the DOQ Priorat (Catalonia, NE Spain), focusing on the grape varietal *V. vinifera* 'Carignan' and 'Grenache'. The availability of data to characterize the climatic variation between small plots is an essential tool for improving crop management under such extreme conditions. These conditions, together with the projections of climatic models, make this region vulnerable to current global change.

Climate classifications are based on the study of different meteorological elements over long periods of time in order to know the general characteristics of the atmosphere in a

given area. Data are calculated and compiled on monthly and annual averages of temperatures, precipitation, etc., and large areas of countries or continents are characterized. Temperature, latitude and precipitation have been used to classify global climates. When defining the climate of a region, reference is made to the macroclimate. In this sense, the Priorat enjoys a temperate Mediterranean climate, like most of the wine-growing areas of the peninsula with the exception of the north, Galicia and Cantabria which belong to the warm temperate oceanic climate; and the Ribera del Duero which has a strong tendency towards continentality. The Mediterranean climate enjoys hot, dry summers, mild winters and heavy rainfall in spring and autumn. However, if we consider the growth of a certain crop such as the vineyard, it is important to know better the climatic particularities that occur in the area and how these affect its annual cycle of growth and fruiting.

The climatic characteristics that come together at the level of a certain plot are called mesoclimates. It is defined in a surface of 10 to 110m. The mesoclimatic differences are more important in the areas where the orography is quite changing, as is the case of the Priorat. The influence of the mesoclimate has a clear effect on the ripening of the grapes and the time of harvest. Also, Alain Carbonneau provides the definition of microclimate, the microclimate at the plant level referring to the conditions of temperature, humidity and insolation that take place inside the vine, at the level of leaves and grapes, which influence the photosynthetic efficiency and consequently in the correct maturation of the grapes.

The mesoclimate determines climatic differences due to the topography of the region and that give rise to local modifications or changes that can affect to more or less ample extensions. Factors that condition them include distance to the sea, altitude, orientation, exposure, and latitude. From one region to another or between nearby municipalities, noticeable differences in temperature, precipitation, insolation and thermal amplitude can be seen, which affect the processes of growth, sprouting, fruit formation, ripening and, ultimately, the composition of grapes. Coastal areas receive the effect of thermal shock absorber from the sea, frosts are rare and summers are rather cool. In contrast, inland winters are very cold and summers very hot, rainfall is scarce, diurnal and annual thermal changes are more abrupt and the thermal amplitude greater. It is said that the climate of these counties tends to be continental. Altitude implies a decrease in atmospheric pressure and consequently a decrease in temperature, an increase in relative humidity, and the possibility of rain. The relief determines not only the climatic conditions by the effect of altitude, but also by the exposure of the earth to the sun's rays which depends on the orientation of the slopes. The conditions of insolation are very different in the sun (cat. *solana*), the slopes that face towards the south, of the shades (cat. *obaga*), slopes oriented towards the north. Depending on the slope of the slope, the rays will be received more perpendicularly in the sun and more radiation will arrive.

In general, all climate change models also predict a variation in the hydrological cycle, reducing precipitation between 10 and 40% (Rosenzwieg and Tubiello, 1997) and modifying its frequency and duration. Thus, a reduction in annual rainfall is expected, especially in the summer months, and a higher incidence of episodes of intense rain. In the Mediterranean region in Catalonia and according to the *Tercer Informe sobre el canvi climatic a Catalunya* (TICCC), annual rainfall in this region will decrease 9% from now on to 2050 and temperature is expected to increase +1.4% (MedECC, 2019).

Another factor is the increase in CO_2 concentration. In general, long-term exposure to high concentrations of CO_2 will reduce stomatal conductance by 25% and consequently respiration (Long et al., 2004), also producing an increase in photosynthesis rates, production and efficiency in the water use. The influence of plant material on key physiological processes related to the efficient use of water by the plant has been demonstrated, from water absorption and stress detection by roots, to by water transport (Alsina et al., 2007; de Herralde et al., 2006; Lovisolo and Schubert, 2006), the modulation of hydraulic and chemical signals between root and leaves (Ren et al., 2007; Christman et al. al., 2007) and gas exchange at the leaf level (Flexas et al., 2007; Soar et al., 2004; Bota et al., 2001). All these processes and those of fruit formation and ripening are modified by the environmental conditions of temperature, humidity (Soar et al., 2006), radiation (Schultz et al., 1998, Jeong et al., 2004) or availability of water (Antolín et al., 2003).

Viticulture is an agri-food sector particularly dependent on climatic and meteorological variations. Episodes of extreme weather such as frost, hail or heavy rains before harvest can cause considerable losses of a specific harvest, while long-term climatic changes can determine changes in the maturation potential and in the style of the wines that a region can produce. Furthermore, wine production is highly adapted to local environmental conditions with the use of varieties and techniques that allow optimum quality production for each specific site. Long-term climatic variations put this balance between varieties - soil - climate at risk (Jones, 2007). According to the prediction models, in vineyards in the Mediterranean area the most limiting factor will be the variation in the rainfall regime and the water availability during the summer. This will make the Priorat are more vulnerable to Climate Change. On the other hand, Grenache is one of the Mediterranean varieties which her great alcoholic yield is characterized by a high accumulation of sugar in the berries during the ripening process. The alcoholic degree attained in their wines tends to be much higher than in Carignan wines. The Atlantic variety Cabernet Sauvignon also raises high amount of sugar in the grapes. Consequently, the increase of Grenache wine production enhances an increase of the alcoholic degree in the wines of the Priorat DOQ (de Herralde et al., 2012). In recent years, the DOQ promoted the new plantings of Grenache and Carignan and also an increasing demand on these wines in international markets.

That is why the knowledge of the autochthonous varieties of Priorat, Grenache and Carignan, and the impact of the adverse climatic conditions, will make this heroic viticulture, based on the orographic conditions with little mechanization, small vineyards sometimes non accessible by machinery, and often organized in terraces, the basis for the quality heritage of Priorat.

Chapter 2. Objectives and Outline

2.1 General Objectives

The general objectives of this project are the generation of new knowledge regarding the effect of water deficits in Grenache and Carignan in key physiological periods (flowering, veraison and fruit ripening); as well as its consequences at the level of synthesis of phenolic compounds in grapes and wine. Deepening the knowledge of the variety, adaptability to edaphoclimatic conditions, availability of water in the soil and the ecophysiological responses of the vine are the object of this study, considering the integration of responses at the plant level and the effect of stress on the composition of the fruit and even going further, evaluating the quality of the final wine.

2.2 Problem Statement

The Grenache and Carignan varieties are widely spread in the wine-growing areas of the northwest of the province of Tarragona, especially in the Terra Alta, Montsant and Priorat Denominations of Origin, where they form the basis of the VQPRD. In 2008, our research group started to study of the cultivars Grenache and Carignan in the wine-growing area of Priorat, where the drought factor and high temperatures lead us to consider this DOQ as a vulnerable Mediterranean wine region in the context of global climate change. In previous research with Grenache and Carignan in the Terra Alta wine region (2006 to 2008), these varieties showed a different kinetic of accumulation of phenolic compounds around harvest that were dependent on the terroir and the vintage. For this reason, one of the elements of greatest interest is the effect of climate and soil in relation to the development of the vine, deepening the study of the effect of annual climate variability, as well as its interaction with the soil and its water reserve capacity. Although water stress favours the synthesis of phenols (determinants of quality in red wines), the extreme weather conditions that occur in the Priorat region can have negative implications for the production and composition of the grape.

One of the quintessential indicators of water stress is abscisic acid. The role of ABA (abscisic acid) in the abiotic stress tolerance mechanisms of the plant has been extensively studied because it significantly limits the productivity of crops of agronomic importance. To assess the water stress in grapevines, a methodology that allows us to give a quick and precise response and that indicates the level of water deficit, together with the measurements of vegetative and productive growth, can allow us to study in depth how the periods of little water availability and high temperatures can affect the synthesis of phenolic compounds that accumulate in fruits. Since some studies show that ABA is involved in the mechanism that controls the synthesis of anthocyanins and intervenes in

the synthesis of tannins that accumulate in the skin, analysis of the phenolic composition by HPLC of the final wine may be essential for the establishment of the quality parameters in relation to water stress.

Secondly, in order to assess the impact of environmental factors and growing conditions on the quality of the wine, it is essential to be able to carry out reliable small-scale fermentations that allow comparable results to be obtained in large-scale commercial vinification. Validating a small-scale methodology is one of the objectives of this project. Researchers often conduct winemaking experiments using small amounts of grapes. Few studies have actually evaluated the efficacy of small-scale fermentations, so it is not known whether reliable and representative data are obtained for replication in large-scale commercial production. Our research would indicate the pros and cons of employing different volumes of small-scale fermentation. Some phenols in wine are released more easily than others. When a sample is not large enough to undergo large-scale fermentation, the total phenols cannot be fully extracted from the wine. This gap would be filled by examining how different volumes could affect the composition of the resulting wines and which would be large enough to conclusively represent a specific winemaking procedure.

2.3 Objectives and their importance

In order to find answers to the proposed objective, three blocks or specific objectives are established:

2.3.1 Chapter 4: Characterization of the Grenache and Carignan varieties in the Priorat and water stress assessment

The characterization studies on Grenache and Carignan allow us to observe if the maturation of the pulp and the seeds depends mainly on the year or the vigour, thus giving rise to a composition of the pulp (probable alcohol, acidity and pH) and phenolic (anthocyanins and tannins) of sufficient concentration to allow us to produce wines with optimal quality parameters. In addition, in some years, the maturation of the pulp will be more advanced than that of the seed and, as a consequence, more heterogeneity and wines with a lower phenolic load. In the case of varieties such as Grenache, a higher sugar content can be observed in warm years. For this reason, vigour management is of great importance to achieve a homogeneous crop to guarantee optimal maturation at harvest time. Therefore, the lack of maturity of the seed in extreme hot conditions and the weak vigour will denote that variety presents a high risk of presenting a negative astringency in the final wines. Based on this knowledge, the objective is to study the influence of the main elements of *terroir* (mesoclimate and soil) and the effect into physiological aspects (vegetative growth, leaf area, water potential, leaf temperature, and harvest production)

and secondary metabolism (accumulation of stress hormones, pulp composition, secondary metabolites). Given the fact that abscisic acid is a good phytohormone indicator of stress, the development of a methodology that can be used in grapevines and that increases the precision and speed in the quantification together with a predictive machine-learning technique that is used for both classification and regression, will allow us to asses water stress and the differentiation of vineyards. The algorithm of the decision tree models repeatedly partitions the data into multiple subspaces so that the outcomes in each final subspace are as homogeneous as possible. This study will be useful to interpret larger data sets from different vineyards and will be helpful to interpret the physiological results obtained.

2.3.2 Chapter 5: Assessment of a small-scale fermentation methodology

At the same time, a small-scale winemaking methodology will be developed in order to assess the repeatability and the reproducibility of the winemaking procedure, that strictly defines the sampling, crushing and distribution of the skin, together with the fermentation conditions, in order to obtain good reproducibility of the method. This will create the basis for establishing a methodology that can be used in subsequent research studies using small-scale fermentation vessels, allowing the results to be extrapolated to a commercial level. This study is of great importance because the availability of grapes in volumes suitable for winemaking is limited in viticulture research and most of the studies related to water stress give rise to an important heterogeneity when orography factors are important.

2.3.3 Chapter 6: Estimated Priorat wine consumption in humans

Phenolic compounds of wine have also attracted much interest due to their antioxidant properties and their potentially beneficial effects for human health. The apparent low bioavailability of anthocyanins seems to cast doubt on their ability to exert their proposed beneficial effects throughout the body. Evaluating within the literature the effects of wine on health, there is no clear evidence of what kind of wine is supposed to have more beneficial effects on metabolic syndrome. Based on recent studies, meta-analysis and pool analysis on wine composition and due to its predicted low bioavailability, it was estimated the efficacy intake of 5 geographically different Priorat wines (Estate Wines), according to recent researches made on gastrointestinal absorption and alcohol intake effect on metabolic syndrome, to better estimate whether geographical origin of wine might have an influence on the daily antioxidant serum composition. The evaluation of different wine/doses let us suggest that the choice of a specific Estate wine in our daily meal would lead to have the same amount of polyphenols avoiding wines with a higher alcoholic degree.

2.4 Justification

Global change is inducing significant variations in the phenology, production and quality of the vine, which strongly depend on the specific region, and which show the vulnerability of this crop and the final quality of the grape and wine facing sustained increases in temperature or significant reductions in water availability. Two important aspects are needed to assess: on the one hand, ecophysiological and genetic variability aspects related to water use efficiency and, on the other, the effects induced by mesoclimatic variations, and specifically of temperature and water availability. The knowledge acquired will make it possible to better assess the vulnerability of viticulture to global change and also identify ways to reduce its presumed impact.

This thesis provides an added value that allows extrapolating the results in the plant and in wine at a commercial level, so that a link of superior knowledge can be reached to validate research studies in the field of vine physiology and micro-fermentations in studies of extraction of phenolic compounds in red wines. The determination of the relationships between ecophysiological parameters and grape composition will allow us to define with greater knowledge both the agronomic potential and the oenological potential of the varieties under study. All this focused on improving certain agronomic practices that guarantee the sustainability of the vine as a crop within the DOQ Priorat and also advise some oenological practices based on the changes in phenolic composition observed in the grapes.

2.5 Limitations and viability

The limitations to this study are the reduced time of the measurements, since in field studies each year the variability of the environmental factors is verified to a lesser or greater degree. Many trials in viticulture need several years of study to draw conclusions about the observed phenomena. In order to partially reduce these limitations, previous studies allowed our research group to evaluate the qualitative potential of the varieties studied in two different wine-growing regions (Terra Alta and Priorat). At the same time, it would be interesting to complete this project with the study of other varieties, in order to be able to integrate all the results and be able to extrapolate to other red varieties.

The proposed work is considered viable due to the integration of previous knowledge, experience and differentiated capacities regarding the experiments to be carried out. The benefits of this project make it possible to assess at the vineyard level, how the cultivation of Grenache and Carignan, contributes to environmental sustainability, in addition to having an impact on the assessment of the quality of the final wine in the DOQ Priorat, but above all to establish methodologies that allow giving validity to similar studies on a commercial scale.

2.6 Previous Publications

Edo-Roca M., Nadal M., **Sánchez-Ortiz A**., Lampreave M. (2014) Anthocyanin composition in Carignan and grenache grapes and wines as affected by plant vigour and bunch uniformity. Journal International des Sciences de la Vigne et du Vin. 48(3):201.

Edo-Roca M., Nadal M., **Sánchez-Ortiz A**., Lampreave M., Valls J. (2014) Vine vigour and cluster uniformity on *vitis vinifera* l. Seed procyanidin composition in a warm Mediterranean climate. Spanish Journal of Agricultural Research 12(3): 772-786.

Nadal M., **Sánchez-Ortiz A**. (2014) Variabilidad Parcelar y maduración fenólica de Vitis vinifera cv. Grenache en viticultura de terrazas en la DOQ Priorato. Enoviticultura. Spanish Wine Magazine. ISSN 2013-6099.

Nadal M., **Sánchez-Ortiz A**., Lampreave M., Edo M., De Herralde F. (2012) Analyse Inter parcellaire de la maturité des raisins et la composition des vins de Carignan dans la DOQ Priorat. 9ème Symposium International d'œnologie, Bordeaux. Editorial: Dunod (http://www.dunod.com/) 255-260. ISBN: 978-2-10-057.

Nadal M., **Sánchez-Ortiz A**. (2011) Territorios de vino: el Priorat. Territoires du vin [en ligne], 03.2011: Los territorios del vino en España. *http://revuesshs.ubourgogne.fr/territoiresduvin/document.php?id=942*. ISSN: 1760-5296.

Nadal M., **Sánchez-Ortiz A**. (2014) Innovacions enològiques els darrers cent anys. Dossiers Agraris, Institució Catalana d'Estudis Agraris, núm . 16 (2013), p . 29-35 ISSN (ed . impresa): 1135-2108 / ISSN (ed . digital): 2013-9772. http://revistes.iec.cat/index.php/DA/DOI: 10 .2436/20 .1503 .02 .40

2.7 Congress participations

Lampreave M., Nadal M., **Sánchez-Ortiz A**., Viñas T., Savé R., De Herralde F. (2010) Producción y composición de la uva en viñas viejas cultivadas en clima mediterráneo cálido (DOQ Priorat). Póster, Congreso Relaciones Hídricas Cartagena.

Nadal M., Lampreave M., Martínez EM., Cancela JJ., **Sánchez-Ortiz A**., De Herralde F., Rey BJ. (2013) Influencia del mesoclima en la estimación del coeficiente de cultivo en viñedos de la DOQ Priorat. Congress GiENOL, Jerez de la Frontera.

Nadal M., Miranda D., Brull A., **Sánchez-Ortiz A**., Mestres M., Busto O. (2013) Influencia de la combinación portainjerto/variedad vinífera en el perfil fenólico de la uva y del vino Gienol. Madrid, 18-21 junio.

Nadal M., **Sánchez-Ortiz A**. (2014) Terroir influence on growth, grapes and Grenache wines in the DOQ Priorat, northeast Spain. X Terroir Viticole Congres. Congres 6-10 June, Tokaj & Eger.

Nadal M., **Sánchez-Ortiz A**., Azuara M., Leriche C. (2014) Impacto del portainjerto y el deshojado en la composición de la uva de Marselan (V. vinifera Grenache x V. vinifera Cabernet sauvignon). I Jornadas del grupo de Viticultura y Enologia de la SECH (Sociedad Española de Ciencias Hortícolas. Logroño 19 y 20 Noviembre.

Nadal M., **Sánchez-Ortiz A**., De Herralde F. (2013) Efectos del cambio climático en la fenología y calidad fenólica de uva y vino en viticultura de terrazas. DOQ Priorato, Tarragona. Gienol, Madrid, 18-21 junio.

Nadal M., **Sánchez-Ortiz A**., De Herralde F., Biel C. (2014) Carignan growth and wine quality influenced by steep slopes and stony soils in the DOQ Priorat, northeast Spain. EGU European Geosciences Union. Vienna 27 April – 02 May.

Nadal M., **Sánchez-Ortiz A**., Lampreave M., Savé R., De Herralde F. (2012) Vintage effect of phenology, yield and wine composition of cv vitis vinifera Carignan in a warm region (DOQ Priorat, Spain). IX Congrès International des Terroirs Vitivinicoles. Dijon (FRANCIA).

Nadal M., **Sánchez-Ortiz A**., Viñas T., Lampreave M., De Herralde F. (2010) Influence of vineyard altitude on the yield and phenolic maturity of Carignan berries in Priorat DOQ. Poster, VIII International Terroir Congress.

Sánchez-Ortiz A., Chávaro-Ortiz M., Nadal M., Martinez-Peniche R. (2014) Composición fenólica de la variedad Marselan (*V. vinifera* Grenache x *V. vinifera* Cabernet sauvignon) bajo el efecto del portainjerto. I Jornadas del grupo de Viticultura y Enologia de la SECH (Sociedad Española de Ciencias Hortícolas. Logroño 19 y 20 Noviembre.

Sánchez-Ortiz A., Nadal M. (2015) Efecto del portainjerto en la variedad marselan adaptada al clima Mediterráneo. Jornadas GIENOL. Tarragona, 6-12 Junio.

Sánchez-Ortiz A., Nadal M. (2015) Impact du porte-greffe sur les composées phénoliques du cépage Marselan en climat Méditerranéen. Symposium OEnologie Bordeaux. (France), 30 june - 2 juillet.

Sánchez-Ortiz A., Nadal, M. (2015) Influence de la variabilité climatique sur le grenache dans le vignoble Méditerranéen. Symposium GiESCO. Bruissan (France), 1-5 Junio.

Sánchez-Ortiz A., Lampreave M., Nadal M., Viñas T., Savé R., De Herralde F. (2010) Influencia del mesoclima cálido en el crecimiento y producción de Vitis Vinifera en la DOQ Priorat. Octubre. Comunicación oral, Congreso Relaciones Hídricas Cartagena.

Santesteban G., Miranda C., Royo J., De Herralde F., Nadal M., **Sánchez-Ortiz A**., López A., Jarén C., Arazuri S. (2013) Evaluation of the variability of skin thickness and berry physical properties in cv. 'Grenache' grown in different areas across the Ebro river valley (Spain). GiESCO, 18e Int. Symposium PORTO, 7-11.

2.8 Publications derived from the Thesis

Sánchez-Ortiz A., Mateo-Sanz JM, Lampreave M, Nadal M (2020) Water stress assessment on grapevines by using a classification and regression trees. Plant direct. 2021:5e00319. htpp://doi.org/10.1002/pld3.319.

Sánchez-Ortiz A., Mateo-Sanz JM, Lampreave M, Nadal M (2020) Evaluation of the repeatability and reproducibility of small-scale fermentation methodology for Tempranillo and Cabernet sauvignon OenoOne: in revision

Sánchez-Ortiz A., Mateo-Sanz JM., Lampreave M., Mateos MS. (2020) Influence of geographical origin of wines on the estimated polyphenol consumption in humans. Trends is food science and technology: pending

Chapter 3. Materials and Methods

3.1 Plant material and site location

3.1.1 Plant material and site location for cv. Carignan

To carry out this research, two locations for Carignan have been selected within the DOQ Priorat. The choice of the two locations responds to the fact of the existence of two clearly differentiated mesoclimates in Priorat: the subzone with a maritime influence in which the thermoregulatory breezes directly affect (municipalities of Porrera and Poboleda) and; the subzone at the other end, which opens towards the Ebro river valley, warmer due to the Priorat orography that prevents the arrival of sea breezes (municipalities of Bellmunt del Priorat, La Vilella Baixa and El Molar) (Nadal, 2002).

Location 1) In the municipality of El Molar (UTM X:810512 UTM Y:4562090) in the DOQ Priorat, the vineyard is located on slopes and river terraces oriented to the south-east south, between 200 and 300 m above sea level. The vineyard is presented on terraces and slopes, the vines are over 50 years old, cultivated in bush and grafted on Rupestris de Lot. The study was performed at two Sites (Site 1 and Site 2) located in an early mesoclimate (El Molar) at different altitudes. Sites of the early region El Molar (EM) were located at: Site 1 (41° 9' 90'' N; 0° 42' 75'' E, elevation 220m) and Site 2 (41° 9' 40'' N; 0° 42' 38'' E, elevation 185m). Three plot replications of each combination vigour/variety were randomly distributed in the vineyards, with each elementary plot consisting of 30 vines. Short pruning is performed. The soils are of colluvial origin, formed by a mixture of slate and calcareous materials, with loam to clay loam textures and with a percentage of stoniness that depends on the level gradient. Harvest dates in this location are traditionally mid-September.

Location 2) In the municipal area of Porrera (UTM X: 320300 UTM Y: 4562075) on a west-southwest facing slope with vineyards between 425 and 495 m above sea level, on terraces with different slopes. The majority variety of *Vitis vinifera* is Carignan, with the presence of some scattered red Grenache. The majority rootstock is R-110 (*V. rupestris x V. berlandieri*) and Rupestris de Lot (*V. rupestris*). Three Sites were selected for the late region in Porrera (PO): Site 3 (41° 10' 51'' N; 0° 52' 25'' E, elevation 425m), Site 4 (41° 10' 50'' N, 0° 52' 29'' E elevation 425m), and Site 5 (41° 10' 57'' N, 0° 52' 32'' E elevation 495 m). Vines are over 60 years old conducted in bush. The soil texture is based on slate, or commonly named as *llicorella*, with a coarse fraction (> 2 mm) 39.7% and a fine fraction (≤2 mm) 60.3% and a loamy texture. The traditional harvest dates in this location are at the end of October. Carignan old bush vines planted in a density of 5000–6000 vines·ha^{-1}. Vines were planted

in steep terraces with a slope of 15–25%. The soils were composed of slate conferring a stony, dry, and poor soil. Furthermore, the soils were well-drained, as they contained a high proportion (between 70% to 90%) of large particles more than 2 mm in diameter. Three plot replications of each combination vigour/variety were randomly distributed in the vineyards, with each elementary plot consisting of 30 vines.

3.1.2 Plant material and site location for cv. Grenache

Location 3) Two Grenache vineyards are analysed here, both grafted onto R110. The plots are located in the townships of El Molar (EM), Site 6 (41° 9' 21.10'' N, 0° 43' 4.08'' E, altitude 210m) and El Lloar (LO), Site 7 (41° 10' 5.64 "N, 0° 43' 17.18'' E, altitude 240m), and studied during two distinctly different vintages: 2010 and 2011. Soils in both are typical of the region, characterized by poor, dry, and pebbly schist. The USDA classification for EM is sandy loam and silty loam for LO, both are of a co-alluvial origin formation. The terraces are naturally located at progressive topographic heights. Grenache vines in LO are 14 years old, and are growing in east-south facing terraces; EM vines are 16 years old and south-facing. Vine spacing is 1.2m and the inter-row distance is 2.5m. VSP trellising (70cm high) and bilateral cordon pruning characterize both vineyards. The Grenache from Lloar (LO) is grafted onto R-110 and distributed in terraces at different levels, conducted in trellises with a height of vegetation between 60-70 cm. Short pruning is performed on all vines and the trellises are conducted in a vertical system in a bilateral cordon. Harvest dates in this location are traditionally mid-September. Three plot replications of each combination vigour/variety were randomly distributed in the vineyards, with each elementary plot consisting of 30 vines.

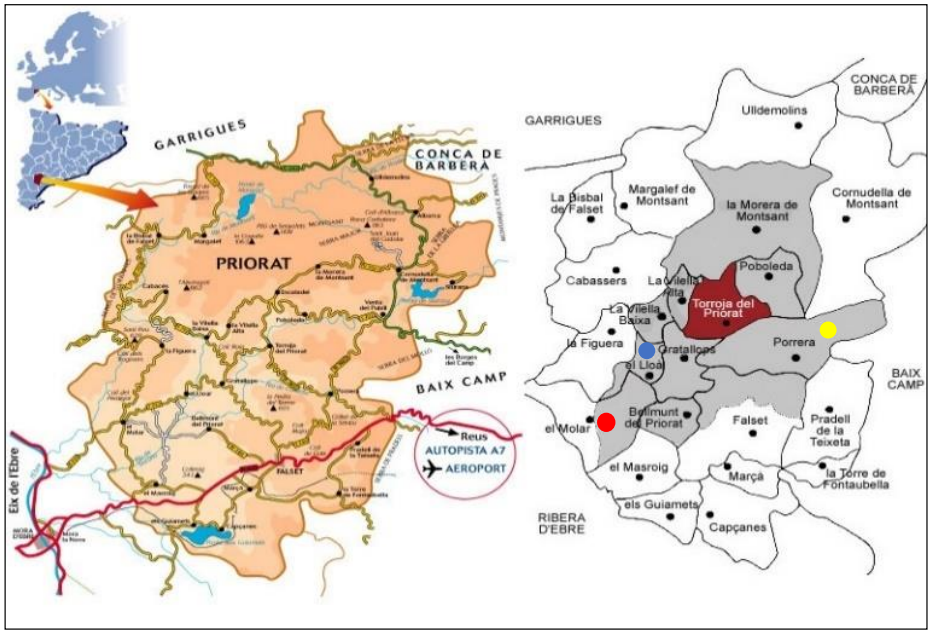

Figure 1. *Priorat county 'comarca' (left) and Priorat wine growing area, DOQ (right). Location of areas of study. EM (El Molar, red point) LO (El Lloar, blue point) and PO (Porrera, yellow point).*

3.2 Climatic characterization

Weather stations (DECAGON®) located in each vineyard recorded various climate data, including temperature (°C), humidity (%), rainfall (mm), and radiation ($W \cdot m^{-2}$). VPD (vapor pressure deficit) was also calculated. Also, the evapotranspiration (ET_0) based on Hargreaves (Hargreaves and Samani, 1985) was calculated with average temperature and radiation (Allen et al., 1998).

***Figure 2**. Installation of weather stations in each vineyard (Site 1 to Site 5). Datalogger (right).*

***Figure 3**. Pyranometer, Anemometer, Pluviometer and humidity and temperature sensors. Adapted by (www.metergoup.com).*

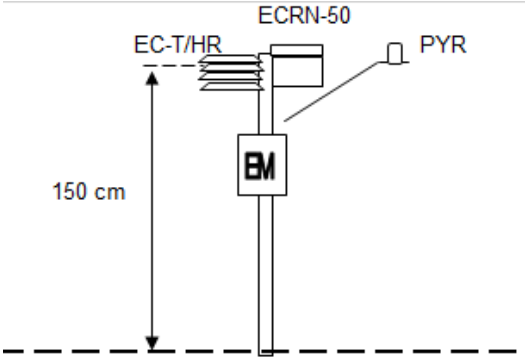

***Figure 4**. Schematic design of a weather station. Model EM5139. Pluviometer (ECRN-50), Humidity and temperature sensors (EC-T/HR)) and Pyranometer (PAR sensor, PYR) (www.metergoup.com).*

Figure 2 shows the installation of Decagon Weather Stations. Each weather station included a pluviometer, pyranometer, anemometer, temperature sensors and humidity sensor. PAR sensors recorded photosynthetically active radiation. Anemometer recorded wine speed and direction.

3.3 Soil characterization

Figure 6 shows the EC-5 soil moisture sensors that were installed in the five vineyards. EC-5 sensor recorded volumetric water content. **Figure 7** shows the picture of the datalogger that allows data storage. The Em50 has 5 sensor ports and a communication port. The Em50 is configured by plugging a laptop into the communications port. The included ECH2O Utility software provides windows setup that will name the logger, set the clock recorder, select the type of sensor on each port, and specify how often you want the sensors to read. The sensors were placed according to the scheme of **Figure 5**. Sensors are installed at three different depths, 20, 40 and 60cm.

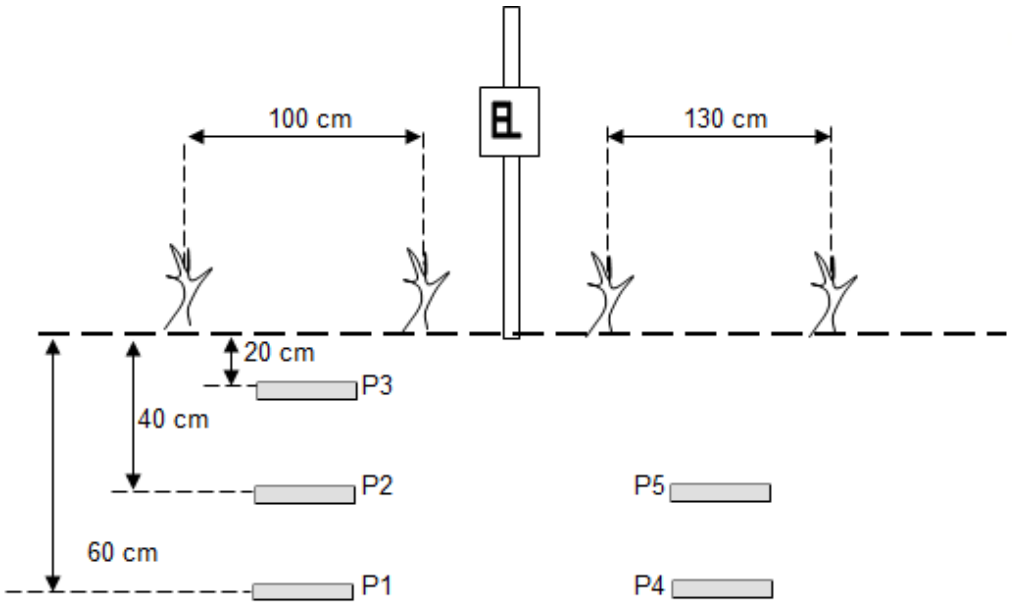

***Figure 5**. Schematic representation of the distribution of Decagon soil moisture sensors. Model EC-5. 5 sensors were installed at different soil depth (P1: 60cm; P2: 40cm; P3: 20cm; P4: 60cm and P5: 40cm) (www.metergoup.com).*

***Figure 6**. Installation of water soil sensors in each vineyard (Site 1 to Site 5). Datalogger. (www.metergoup.com).*

***Figure 7**. Datalogger (left) and ECH2O EC-5 Soil moisture sensor (right) used to determine soil humidity. Every datalogger can connect up to 5 sensors (www.metergoup.com).*

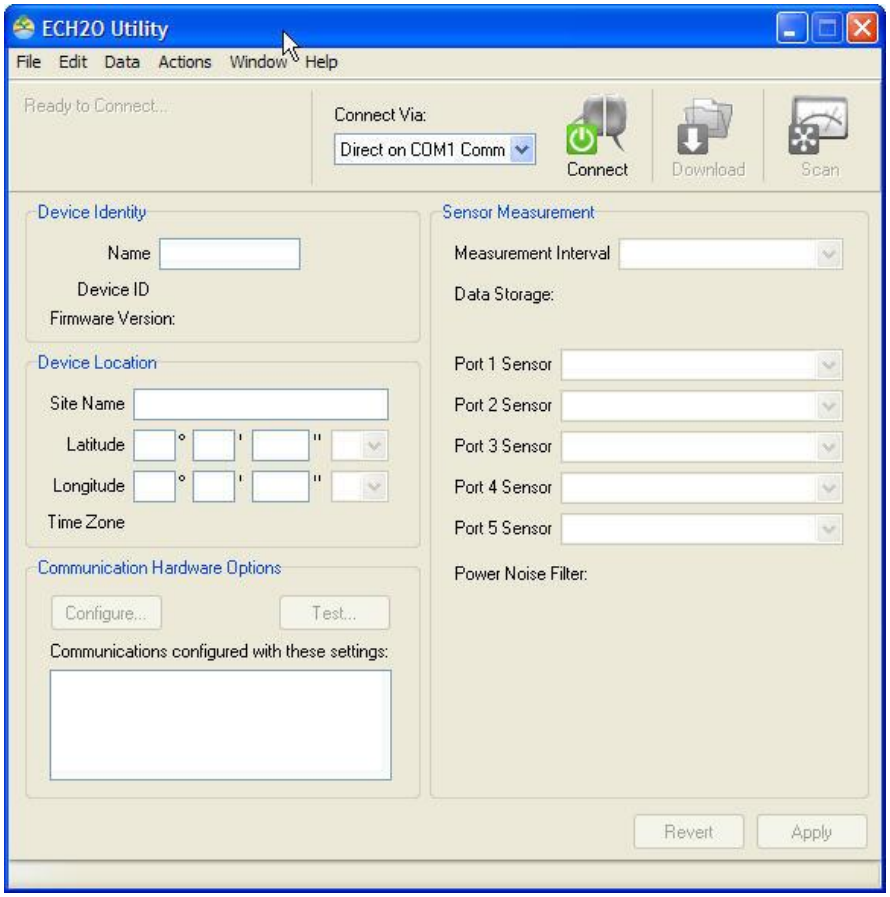

***Figure 8**. ECH2O Utility software. Every datalogger was identified by name and location that was determined by Latitude and Longitude. 5 sensors were plugged at each port (P1: 60cm; P2: 40cm; P3: 20cm; P4: 60cm and P5: 40cm.*

Regarding geology, the oldest materials date in the Priorat of primary geological era, 400 million years ago in the Palaeozoic and during different periods (Devonian, Carboniferous, Permian). More recent in the Primary, the Carboniferous era, the slate appears to make up the majority of the soils of Priorat. The soils have different colours and brightness depending on the type of minerals and oxides are formed and cemented with sand: brilliant black, brown, red, grey and freckled. Hercynian movements of Mesozoic cause an uprising of a whole set of substrates, formation of new sediment and cement will result in the red sandstones that are so characteristic of today's towns. Finally during the Quaternary glacial periods and the alternation of interglacial impact on the erosive power of water currents generated on the territory the formation of fluvial terraces and deposits rates glaciers that have come to shape the today's relief.

In the Priorat, there are three types of soils of different origin:

a) **Calcareous soils**: These are soils formed by materials from the Tertiary, they are silts that come from limestone that form the mountains of the area. These soils are located in the Montalts and Montsant and more specifically in plots of

Morera del Montsant and El Molar. These soils are deep with a more or less developed petrocalcic.

b) **Llicorella (slate) soils**: These are soils that develop on Palaeozoic shales, usually from the Carboniferous. Decomposed slate layers alternate with other siliceous materials and sometimes with the presence of calcium cements. In addition these slate soils are the result of the consolidation of clayey sediments. These are the most important in the DOQ Priorat and make up the typical landscape of this region. These soils are located in the locations of La Viella Baixa, La Viella Alta, Gratallops, El Lloar, Torroja, Poboleda and Porrera.

c) **Granitic soils**: These soils are formed by materials from the pre-Cambrian period, these materials are very decomposed granites. These are sandy soils located in flat areas around the towns of Falset, Marçà and some can also be found on land in Bellmunt and in the direction of Gratallops.

Mostly, within the DOQ Priorat, we find three different types of soils, two of which belong to the Palaeozoic period while another belongs to plutonic and hypabyssal rocks.

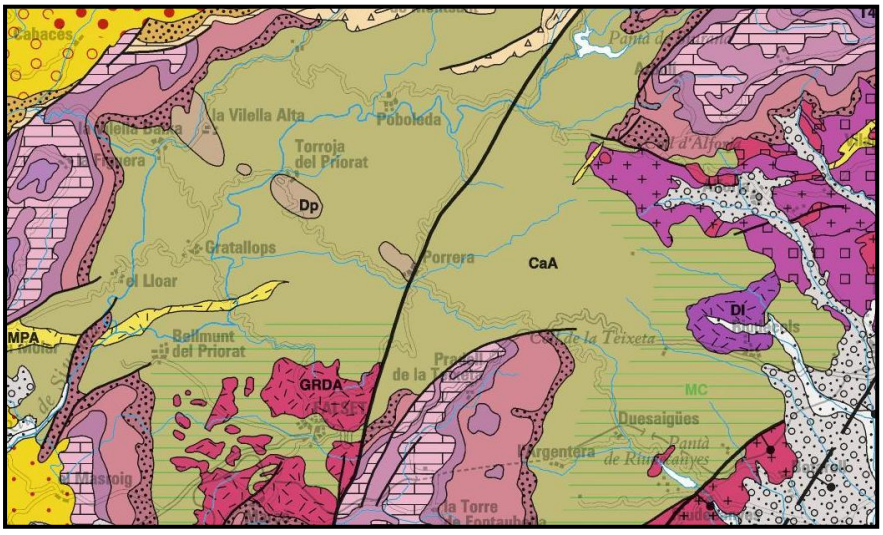

Figure 9. *Priorat geological map (ICC:Institut Cartogràfic de Catalunya). Scale 1:250.000.*

There are three geological periods within the study area:

a) **Palaeozoic-Devonian***:* This is the oldest soil of the DOQ Priorat. This period is from 397 million years ago to about 359 million years ago. In this case, the general characteristics of this soil are clayey slates with intercalations of quartzites and lyddites predominate.

b) **Palaeozoic- Carboniferous***:* The soil of this period is the most extensive in the DOQ Priorat. The Carboniferous goes from 359 million years ago to about 326 million years ago. In this case, the most general characteristics of these types of soils are that sandstones and slates with conglomerate levels predominate, with andesites at the base.

c) **Plutonic and hypabyssal rocks***:* Late Hercynian intrusive rocks: These rocks are classified in igneous rocks, they derive from the crystallization of a magma, and the process that generates them is magmatism. Hypabyssal and subvolcanic rocks are formed by solidification of magma within cracks or fractures, forming dikes and sills. Plutonic rocks are present in masses, usually large in size, depending on their shape and dimensions laccolites, phacolites, lopoliths or batholiths will form.

From the geological point of view, in the Priorat the soil that stands out most is that of *Llicorella*. This soil absorbs and stores moisture giving the vineyard optimal soil. In addition, the slate holds the heat radiated by the floor, and at the same time reflects light. All these characteristics make the wine obtained have a very personal and typical character of the area.

Agronomically speaking, the soils of the DOQ Priorat are stony, sandy and relatively unfertile due to their poverty in terms of organic matter. The metamorphic nature of the stony elements facilitates the breakage of slate in the direction of the layers of stratification, the outcome of which is the formation of flat *Llicorella* (slate) stones that cover the surface of the soil. On the slopes, these flat slate stones contribute to diminishing the magnitude of erosion phenomena that would normally occur on such steep slopes.

Slate (in Catalan *Llicorella*, with local variations such as *licorella*, *llicorell* or *llecorell*), is the unchallenged protagonist od the DOQ Priorat, although the region also comprises a number of areas from which slate is absent, such as the foothills of the Montsant and much of the mountain itself. The term *Llicorella* is linked to *Llècol*, a word used to designate humour, taste, flavoursome mellowness, the etymological source of which is the Celtic *Likka*, which means stone (Lopez-Monné et al., 2004)

3.4 Vegetative growth

Two vines per replicate were used for vigour measurements, with a total of six vines per treatment. Number of shoots per vine, diameter and length of shoots, clusters per vine, berry weight, bunch weight, yield, pruning weight and total leaf area were measured in each replicate. Furthermore, Ravaz index (yield/pruning weight) and the ratio of the total leaf area TLA/yield were calculated.

Individual 100 leaf areas were scanned by a CI-202 Leaf Area Meter. The CI-202 Laser Area Meter uses advanced laser technology to provide with a precise and convenient way to measure leaf area. The high-resolution laser scanner, data logger, and display are all enclosed in a durable, handheld scanner and detachable palette. The CI-202 is used to perform non-destructive measurements on the leaves of living plants by placing the leaf on the palette and sliding the scanner over the leaf, enabling collection of data from the same plant, or even the same leaf, throughout its life span. The transparent, protective sheath on the palette makes it easy to capture precise leaf area measurement on tender or intricate leaves. The total leaf area was calculated by using the methodology described in Edo-Roca et al. (2014).

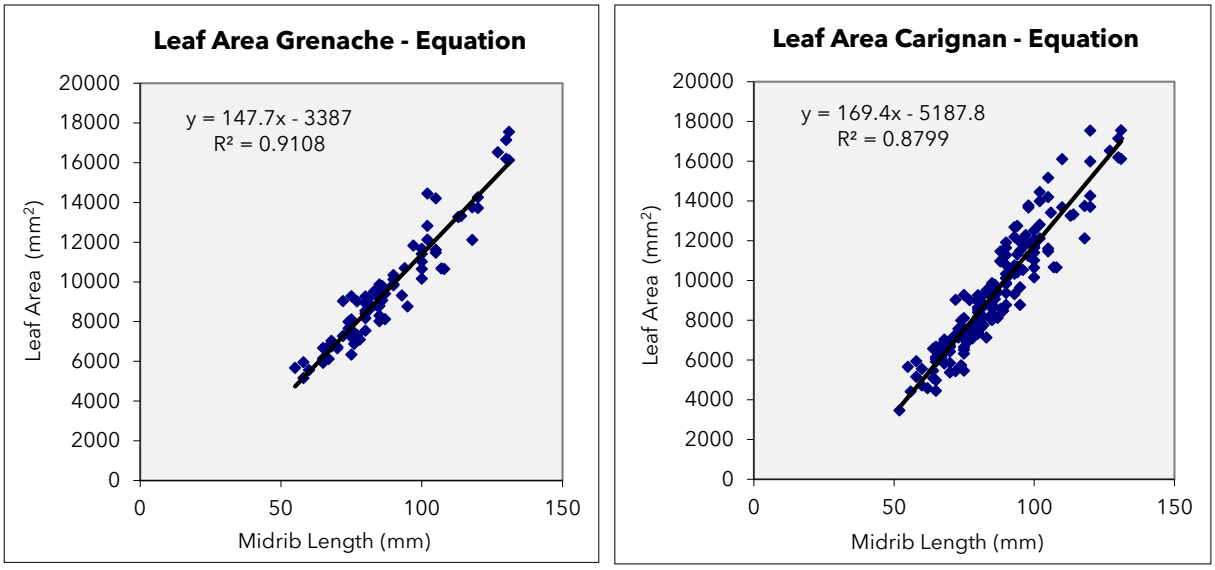

***Figure 10**. Equations used to calculate TLA for cv. Grenache and cv.Carignan.*

After scanning the leaf area we proceed with measuring the length of the midrib (central nerves), which correlated well with the area of the leaf. The equation used for cv. Grenache was y=147.75x-3387 (R^2=0.9108) and for cv. Carignan y= 169.43x-5187.8 (R^2=0.8799).

3.5 Phenology

Some of the most clearly observable effects of climatology on plant biology are summarized in phenological observations, already in the key dates that define the annual cycle of the species, either in those quantitative parameters that we consider phenological. The different phases of growth and development of the vine are defined in 16 phenological stages, each of them being recognized by a letter according to the methodology described by Baggiolini (1952) and by Lorenz et al. (1995). It is established that the full phenological state is reached in the count of 50% of elements in a certain phenological state. In our study, it was of great importance to know the duration of each stage under the influence of the mesoclimatic variable and to determine the percentage of accumulation in each stage. In three vines of each elemental plot, the dates of sprouting, full flowering, fruit set, pea size berry, veraison, harvest (optimal ripening) and leaf fall was determined. The number of buds per vine, the phenological stage and the percentage of each stage was calculated.

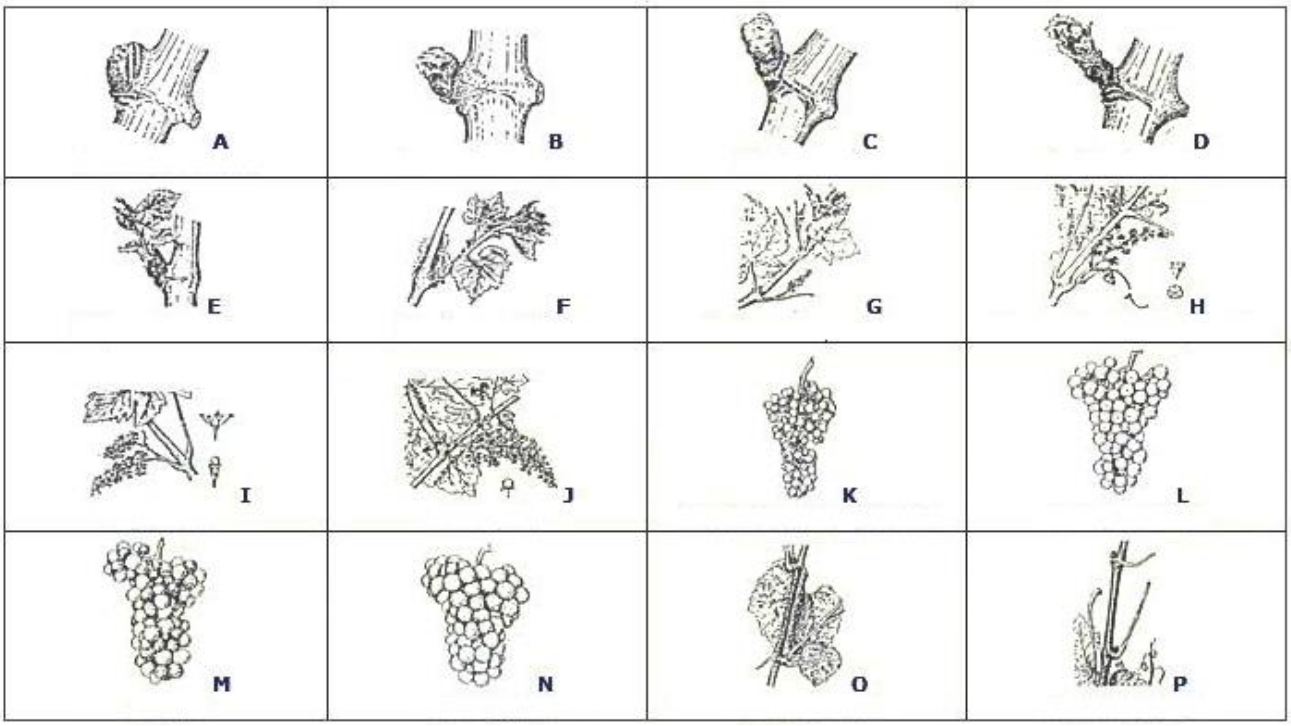

Figure 11. *Phenological stages by Baggiolini: **A** (winter dormancy), **B** (wool, doeskin stage), **C** (green shoot), **D** (first leaf unfolded), **E** (2 to 3 leaf's unfolded), **F** (inflorescence clearly visible), **G** (inflorescence elongating, flowers closely pressed together), **H** (inflorescence fully developed, flowers separating), **I** (full flowering, 50% of caps falling), **J** (fruit set, young fruits beginning to swell), **K** (berries pea-sized, bunches hang), **L** (beginning of berry touch), **M** (beginning of berry ripening, loss of green colour, veraison), **N** (ripeness), **O** (lignification), **P** (leaf falling).*

The budburst represents the starting point of the plant growth with the appearance of the first leaves. From this moment on, the plant will, once again, start its photosynthetic activity and shift progressively from a growth based on its reserves to a growth based on the

production of newly synthesized carbohydrates. This is the methodology used for the determination:

For notations:

- We take into account only the vine-plants that are definitively established and in production.
- We recognize that a bud is in budburst if we see a small green or red tip.
- We consider only the principal buds.
- The retained stage corresponds to the date at which 50 % budburst has been reached in relation to the number of productive buds left at the pruning.
- It is necessary to undertake the observations on at least five vine-plants per homogenous zone

Passage frequency:

- From the moment when a minimum of 5 % of buds have burst, at least one additional passage was done with a maximum of one-week interval, in a manner to have one observation after 50 % of the buds have burst.
- The date of «50 % budburst» is obtained by interpolation between the observed values before and after 50 %.

The flowering marks the beginning of the reproductive stage: the fall of the cap corresponds to the moment where the pollen will come into contact with the stigmas. The process of fertilization of the ovum that follows, conditions the formation of the berries and the pips, it thus constitutes a crucial moment in the development cycle.

For notations:

- Take into account only the vine-plants that are definitively established and in production.
- It is considered that a flower is open when the base of the cap is detached, regardless of whether it falls off or not.
- We estimate a level of flowers open. The retained stage corresponds to the date at which a level of 50 % is reached.
- It is necessary to undertake the observations on at least five vine-plants per homogenous zone.
- To determine the stage of 50 % flowering, we evaluate the level of flowering per vine-plant or by inflorescence, then we calculate an average.

Passage frequency:

- From the moment when we observe a minimum 5 % of flowers open, do at least one supplementary passage with a maximum of one-week interval, in a manner to have one observation after 50 % of the flowers have opened.
- The date of «50 % of flowers open» is obtained by interpolation between the observed values before and after 50 %.

The veraison marks the beginning of the ripening process of the grapes, that finishes at the harvest.

For notations,

- Take into account only the vine-plants that are definitively established and that are in production.
- We consider that a berry has completed its veraison if it is soft to the touch.
- This criterion permits an unbiased comparison of the grape varieties, whether white or red.
- Always undertake notations at the same hour, preferably in the morning.
- The retained stage corresponds to the moment at which the berries are soft to the touch.
- The use of the colour appearance method is acceptable for interannual comparisons of the same grape variety at the same site. In this case, a visual estimate of the percentage of coloured berries on the entirety of the bunches of the vine must be effectuated.
- It is necessary to undertake the observations on a minimum of five vine-plants per homogenous zone.

Passage frequency:

- From the moment when we have observed a minimum of 5 % of berries soft to the touch, we did at least 1 supplementary passage with a maximum of one-week interval, in a manner to have one observation after 50 % of the berries are soft to the touch.
- The date of «50 % berry veraison completion» is obtained by interpolation between the observed values before and after 50 %.

3.6 Leaf water potential, Ψ_{LWP} and stomatal conductance, g_s

Measurements of stomatal conductance (g_s) and leaf water potential (LWP) was intended to determine the variations in plant physiology between plots in the same area, potentially attributable to mesoclimatic variations. To perform the measurements of stomatal conductance the SC-1 Decagon Porometer (Decagon Devices, Inc. 2365 NE Hopkins

Court Pullman, WA 99163) was used. This mobile instrument was very easy to transport due to the difficulty of the terrain in the Priorat. Measurements with the SC-1 Decagon Porometer was restricted to stomatal conductance and leaf temperature. Stomatal conductance (mmol m^{-2} s^{-1}) measured by a porometer is the rate of CO_2 entering, or water vapor exiting through stomata. Stomatal conductance (g_s) is a measure of the degree of stomatal opening and can be used as an indicator of plant water status. Stomatal conductance is related to LWP by feedback processes. Reductions in g_s prevent further decreases in LWP by reducing transpiration; also, reductions in LWP can induce stomatal closure, resulting in lowered g_s.

The LWP (Leaf Water Potential) in each phenological stage, PS (pea size), V (veraison), and RP (ripeness), were measured using a pressure chamber (207 Bar/3000 PSI pressure) (Model 600 PMS Instruments, Oaklands Park, Wokingham, United Kingdom) according to the technique described by Scholander et al. (1965). Leaf water potentials are reference measures of vine water status that have enabled solid reference thresholds of vine water status to be established. To ensure consistent readings, predawn LWP (Ψ_{PLWP}) was measured one to two hours before sunrise at 8:00 (6:00 solar time), when grapevine water status is at a maximum (Carbonneau,1998), and midday LWP (Ψ_{MLWP}) was measured at 2:30 (12:30 solar time). In addition, primary (PLA) and secondary leaf (SLA) areas were measured during the PS, V, RP, and PH (postharvest) stages.

***Figure 12**. Infrared thermometer Testo© allowed the measurement of leaf temperature (left). 600 PMS Scholander chamber (right) allowed the measurement of leaf water potential (Ψ_{LWP}) (right)*

***Figure 13**. The steady state SC-1 Decagon ® Leaf Porometer consists of a hand-held enclosure with a cable connected to a leaf-clip sensor. The final reading appears on the display in terms of either conductance or resistance. Saved data can download to a PC using an RS232 cable and download to a PC using an RS232 cable and download utility software. (www.metergoup.com)*

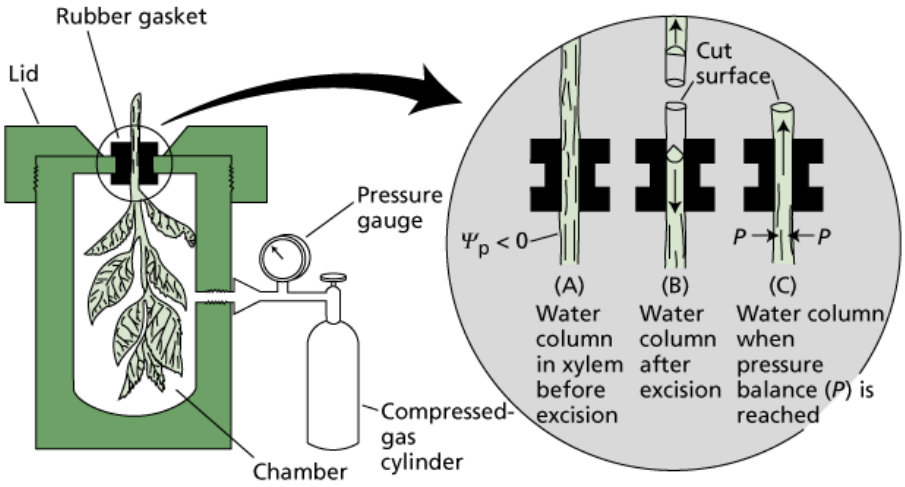

***Figure 14**. The pressure chamber method for measuring plant water potential. The diagram at left shows a shoot sealed into a chamber, which may be pressurized with compressed gas. The diagrams at right show the state of the water columns within the xylem at three points in time: (A) The xylem is uncut and under a negative pressure, or tension. (B) The shoot is cut, causing the water to pull back into the tissue, away from the cut surface, in response to the tension in the xylem. (C) The chamber is pressurized, bringing the xylem sap back to the cut surface. Adapted from: Plant Physiology and Development, Sixth Edition by Lincoln Taiz, Eduardo Zeiger, Ian M. Møller, and Angus Murphy, published by Sinauer Associates.*

3.7 Sample leaf preparation for ABA determination

Several long and tedious methods have been developed for the extraction and determination of ABA in plant tissue; however, some studies have developed more rapid approaches for the determination of phytohormones in plant material other than vine leaves (Riov et al., 1990; Setha et al., 2005). However, the establishment of a rapid method for determining ABA in vine leaves (López-Carbonell and Jáuregui., 2005), along with

measurements of LWP, provided important information for the classification of the water status of the vineyards.

Healthy leaves having reached approximately two-thirds of their definitive size were sampled from five vines per block and were bagged using Ziploc ® bags covered with a metalized high-density polyethylene reflective film to avoid additional leaf heating. This approach prevents the degradation of phytohormones, such as ABA. Samples were stored at -20ºC. The methodology of López-Carbonell et al. (2009) was used for the extraction of ABA in Carignane leaves. Extraction solvent (solution 1) was prepared with acetone/water/acetic acid (80:19:1, v/v/v). The solvent temperature was kept at -20ºC. Reconstitution solvent (solution 2) was prepared with water/acetonitrile/acetic acid (90:10:0.05, v/v/v).

This methodology was improved by carefully weighing 4–5 g of fresh weight from a pool of different leaf samples and lyophilizing samples in a Telstar LyoQuest freeze dryer with a condenser temperature of -55ºC, followed by powdering with mortar and pestle. Dried samples were carefully weighed in a 1.5-mL Eppendorf tube. Next, 1 mg of ABA internal standard was added to each of the three replicates at the beginning of the extraction procedure. A volume of 1.2 mL of extraction solvent (solution 1) with the 300 mg of sample inside the Eppendorf was extracted in triplicate, and temperatures remained cool while samples were manipulated. The Eppendorf mixture was vortexed and left overnight at -20ºC, followed by centrifugation at 15,000 rpm for 10 min at 4ºC. Supernatants were pooled, dried under a nitrogen stream (Stuart, SBH200D), and reconstituted in 445 µL of reconstitution solvent (solution 2), followed by stirring, vortexing, and centrifugation (10,000 rpm, 10 min). Samples were filtered through a 0.22-µm PTFE filter (Millex Syringe-driven Filter Unit). Next, 5 mL of each sample was injected into the LC–ESI–MS/MS system. Internal standards were used for the calibration of ABA. The calibration curves for ABA showed high linearity (R^2 = 0.9959). The regression equation for the relationship between area (EIC) and ABA concentration (mg/L) was ABA = 1×10^6Area - 138.14. ABA standards were prepared daily. High correlation coefficients (r^2 > 0.995) were obtained for concentrations ranging from 0.019 to 0.472 mg/L.

3.8 Fruit sampling and analysis

Berry ripening was carefully monitored, and chemical analyses of the resulting wines were evaluated. During harvest, weekly samples of 400 berries were randomly harvested and then analysed. Sugars (Brix), TTA (g/L total titratable acidity), and the pH of the grape juice were determined. After crushing the whole berries, extraction of phenolic compounds was performed following a modified version of the Glories method (Nadal, 2010) to determine total anthocyanins (ANT T) and extractable anthocyanins (ANT E); %EA (extractability of

anthocyanins), %SM (seed maturity), and TPI (total polyphenol index) were also measured. OIV methods were used to analyse alcohol by volume (ABV), total titratable acidity (TTA), pH, anthocyanins, DMAC (flavan-3-ol by derivatization with p-*dimethylaminocinnamaldehyde*), and total tannins in wine. ANOVA was performed using the general linear model procedure. The Tukey test was used for *post-hoc* analysis (XLSTAT statistical package, EXCEL) between plots. The evolution of grape ripeness and wine composition at the chosen Sites was followed at each of the municipalities. Small-scale fermentations were performed for each Site in triplicate. Grapes were randomly sampled, de-stemmed, crushed into stainless-steel wine vats, and fermented after 3 days of cool maceration to extract the colour and following the fermentation of all sugars. Potassium metabisulfite was added to a final concentration of 20 ppm to preserve the products of oxidation processes until bottling. The wine did not undergo malolactic fermentation. The composition of wine was determined at all Sites. Specifically, alcohol by volume (ABV, OIV), total acidity (TTA, OIV), pH (OIV), total anthocyanins (Ribéreau-Gayon et al., 2003), tannins, and flavan-3-ol (DMAC method developed by Vivas et al., 1994) were determined.

3.9 Winemaking procedure

Grapes were handpicked at full ripeness into 20kg boxes, and stored at 21°C in a cold room before crushing. Grapes were de-stemmed and crushed individually for each tank volume using a BucherVaslin® Delta E2. Tanks were filled one-by-one to three-quarters capacity in order to ensure an upper appropriate fermentation cap management. Room temperature during fermentation was kept at 23°C, and 40mgL^{-1} sulphur dioxide was added to the must. All tanks were inoculated with 0.2gL^{-1}yeast (ICV GRE Selection Inter Rhône, Lallemand®). The pomace was gently hand-punched down twice a day until alcoholic fermentation was accomplished. During the tumultuous stage, must density and temperature were both measured daily, controlling sugar consumption, and avoiding extremely high temperatures (higher than 28°C) during the winemaking process. The pomace was pressed once fermentation was completely exhausted (reducing sugars <2gL^{-1}). Free-run wines were then obtained using a cone-shaped funnel (Lacor inox 18/20; diameter 22cm) to separate the pomace from the wine. Press wine was obtained using a 40L Hydropress with a capacity of juice yield of up to 20–25L per pressing, depending on variety and ripeness of fruit (http://www.vigopresses.co.uk). After pressing, the juice was settled overnight and racked to the same tank to promote clarity. Potassium metabisulphite was added (Winy Sepsa Enartis®) to reach 20mgL^{-1} of sulphur dioxide to prevent microbial spoilage. Wines were stabilized at 4°C for 2 months, followed by racking before bottling, and kept at 4°C for further storage. Finished wines were bottled without fining or filtering. The wines did not undergo malolactic fermentation to avoid unwanted apparent malolactic deviations, and no oak treatment or aging was undertaken.

3.10 Determination and identification of anthocyanins and procyanidins by RRLC-DAD-TOF/MS.

All solvents were of HPLC grade. Water, methanol and trifluoroacetic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Standard of malvidin-3-*O*-glucoside was purchased from Sigma Aldrich (St. Louis, MO, USA).

Grapes and wines preparation: The phenolic maturity of grapes was analysed according to the modified Glories method (Nadal, 2010). The extract (at pH =1; total anthocyanins) was previously filtered by using PVDF (0.22µm) before carrying out the analysis of anthocyanins by (RRLC-DAD-TOF/MS). The same Liquid Chromatography procedure was followed for the wine samples.

Instrumentation: Anthocyanin content was determined following the methodology detailed in Valls et al., (2009) and adapted from Deviliers et al. (2004) through high-performance liquid chromatography (HPLC) using a Hewlett Packard Liquid Chromatograph (Waters Corporation, Mildford, MA, USA) equipped with a Zorbax Eclipse Plus C_{18} Column (150×2.1mm; 3.5µm) and a Zorbax Eclipse Plus-C18 Precolumn (12.5×4.6mm; 5µm). Injection volume was 5µL; elution was performed with a mobile phase A of HPLC-grade water (0.2% trifluoroacetic acid) and a mobile phase B using methanol (0.2% trifluoroacetic acid). The column temperature was set at 50ºC. The HPLC was coupled to a Diode Array Detector (DAD) (Peak width > 0.1 mm (2s); storage of all 190–700 nm step 2 nm; slit 4 nm; margin for negative absorbance 100 mAu. *ITMS conditions*: ionization source ESI positive; ion trap analyser (capillary 3500 V, target mass 493 m/z, comp stability 100%, trap drive level 100%, scan 100–900 m/z, ICC smart target 500000, max accu time 200 ms, average 5).

The anthocyanidin mono glucosides of the wines were chromatographed by HPLC using a Beckman Ultra sphere (C_{18}) ODS (250 × 4.6 mm i.d.) column, and detection was carried out at 520 nm. The solvents were A, H_2O/HCOOH (9:1), and B, CH_3CN/H_2O/ HCOOH (3:6:1). The gradient was 20–85% B for 70 min, 85–100% B for 5 min, and then isocratic for 10 min at a flow rate of 1 mL/min. The content in free anthocyanins was determined using a calibration curve (based on peak area), which was established using malvidin 3-glucoside.

Quantifications were performed using the DAD detector, and identifications were made considering the time of flight (TOF). A mass spectrometry (MS) detector was used to assist in the identification. The contents of free anthocyanins were determined using calibration curves (based on peak area), which were established using malvidin 3-glucoside

(Extrasynthèse®). Standard solutions were subjected to the same procedure (y= 0.7968x + 7.5756, R^2=0.9774). The anthocyanidin-3-monoglucosides and respective acetylated and coumaroyl glycosides were identified based on their UV-Vis spectra and retention times. The anthocyanidins were identified by HPLC by comparison with internal standards. The calibration curves were obtained by injecting standards with different concentrations of malvidin 3-glucoside (Sigma). The range of linear calibration curves was from 0.1 to 1.0 mg/L for the lower concentration compounds (R^2 > 0.996), 0.1 to 5.0 mg/L for intermediate concentration compounds (R^2 > 0.987), and 10.0 to 200.0 mg/L for the higher concentration compounds (R^2 > 0.987). Unknown concentrations were determined from the regression equations, and the results were expressed in mg of malvidin 3-glucoside per berry. Repeatability of this method from extraction to HPLC analysis for four samples of the same batch of grape skins had a coefficient of variation < 7%.

Triplicates from each sample were analysed. The different phenolics compounds analysed were tentatively identified according to their order of elution, retention times of pure standards (catechin, epicatechin, catechin gallate, epicatechin gallate, procyanidin B1 and B2) (Fluka). Anthocyanin quantification was made using the calibration curves belonging to the most similar compound: malvidin-3-glucoside. Total amount of anthocyanins was given in mg/g berry (grapes) and mg/L (wines).

3.10.1 Chromatographic conditions for anthocyanin analysis

Table 1 shows the retention times for the anthocyanidins (glucosides, acetilglucosides and cumarilglucosides). **Figure 15** shows the chemical structure of anthocyanins with different R groups (cyanidin, delphinidin, peonidin, petunidin, malvidin).

***Table 1**. Peak assignments, retention times and mass spectral data of anthocyanidins. glucoside (1 to 5), acetyl glucoside (6 to 10) and coumaroyl glucoside (11 to 15).*

	Anthocyanin	Retention	M^+	M^+-X	Transition
1	Delphinidin 3-O-glucoside	10.8	465	303 (M^+-glu)	465→303
2	Cyanidin 3-O-glucoside	11.8	449	287 (M^+-glu)	449→287
3	Petunidin 3-O-glucoside	12.5	479	317 (M^+-glu)	479→317
4	Peonidin 3-O-glucoside	13.4	463	301 (M^+-glu)	463→301
5	Malvidin 3-O-glucoside	13.8	493	331 (M^+-glu)	493→331
6	Delphinidin 3-O-acetilglucoside	15.3	507	303 (M^+-gluAc)	507→303
7	Cyanidin 3-O-acetilglucoside	16.2	491	287 (M^+-gluAc)	491→287
8	Petunidin 3-O-acetilglucoside	16.7	521	317 (M^+-gluAc)	521→317
9	Peonidin 3-O-acetilglucoside	17.6	505	301 (M^+-gluAc)	505→301
10	Malvidin 3-O-acetilglucoside	17.8	535	331 (M^+-gluAc)	535→331
11	Delphinidin 3-O-cumarilglucoside	17.6	611	303 (M^+-gluCou)	611→303
12	Cyanidin 3-O-cumarilglucoside	18.5	595	287 (M^+-gluCou)	595→287
13	Petunidin 3-O-cumarilglucoside	18.7	625	317 (M^+-gluCou)	625→317
14	Peonidin 3-O-cumarilglucoside	19.3	609	301 (M^+-gluCou)	609→301
15	Malvidin 3-O-cumarilglucoside	19.4	639	331 (M^+-gluCou)	639→331

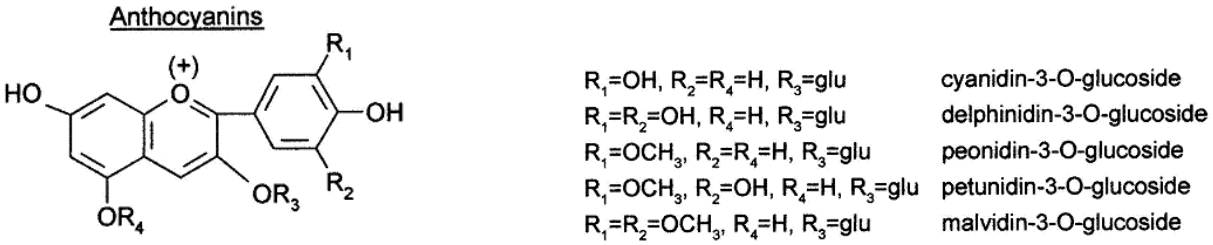

***Figure 15**. Anthocyanin structure.*

3.10.2 Chromatographic conditions for procyanidin analysis:

The procyanidin extracts were chromatographed and detected by HPLC-DAD using two connected columns MERCK (C_{18}) ODS (250 x 4.6 mm i.d.) protected with a guard column packed with the same packing according to the procedure described elsewhere (De Freitas and Glories, 1999). The elution system consisted of two solvents, A: 2.5% acetic acid in water, and B: 80% acetonitrile in A. Gradient elution consisted of: 7% B with isocratic elution for 5 min; 7-20% B, from 5 to 90 min; 20-100% B, from 95 to 100 min; 100% B, from

100 to 110 min (isocratic), followed by washing and reconditioning of the column. The analysis was carried out at 25°C and at a flow rate of 1.0 mL/min. The procyanidin dimers B1 to B8 and trimer C1 used as standards were synthesised following the reported methods (Geissman and Yoshimura, 1966; Michaud *et al*., 1973). Procyanidin dimers in grape extracts were identified by analytical HPLC, by comparison with authentic standards (De Freitas *et al*., 1998b; Rigaud *et al*., 1991). The (-)-epicatechin *O*-gallate and B2-3''-*O*-gallate were collected from the HPLC column and their structures were elucidated by NMR (De Freitas, 1995).

Table 2. *Retention time and m/z for each compound.*

	Procyanidin	Retention time	(m/z)
1	Procyanidin C (trimer)	0.6	865.1989
2	Gallic acid	0.8	169.0147
3	Procyanidin B3 (dimer)	1.9	577.1364
4	Procyanidin B1 (dimer)	2.1	577.1364
5	Procyanidin C (trimer)	2.4	865.1989
6	Catechin	2.8	289.0722
7	Procyanidin B4 (dimer)	3.4	577.1364
8	Procyanidin B2 (dimer)	3.7	577.1364
9	Dimer monogallate	4.5	729.1469
10	Dimer monogallate	4.7	729.1469
11	Epicatechin	5.0	289.0722
12	Procyanidin C1 (trimer)	5.0	865.1989
13	Procyanidin B (dimer)	5.1	577.1364
14	Dimer digallate	5.7	881.1683
15	Epicatechin gallate	6.2	441.0835
16	Procyanidin B (dimer)	6.6	577.1364

Compounds determined: Procyanidin C (trimer), Gallic acid, Procyanidin B3 (dimer), Procyanidin B1 (dimer), Procyanidin C (trimer), Catechin, Procyanidin B4 (dimer), Procyanidin B2 (dimer), Dimer monogallate, Dimer monogallate, Epicatechin, Procyanidin C1 (trimer), Procyanidin B (dimer), Dimer digallate, Epicatechin gallate, Procyanidin B (dimer). After carrying out HPLC for procyanidin content in wine, table 2 shows the retention time and *m/z* for each compound.

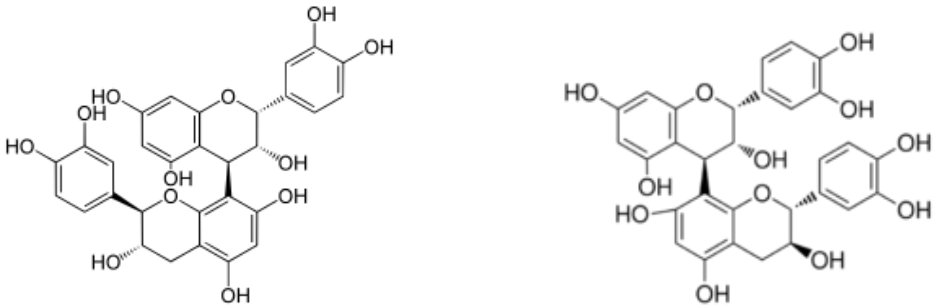

Figure 16. *Procyanidin B1(left) and Procyanidin B2 (right). Adapted from PubChem (pubchem.ncbi.nlm.nih.gov).*

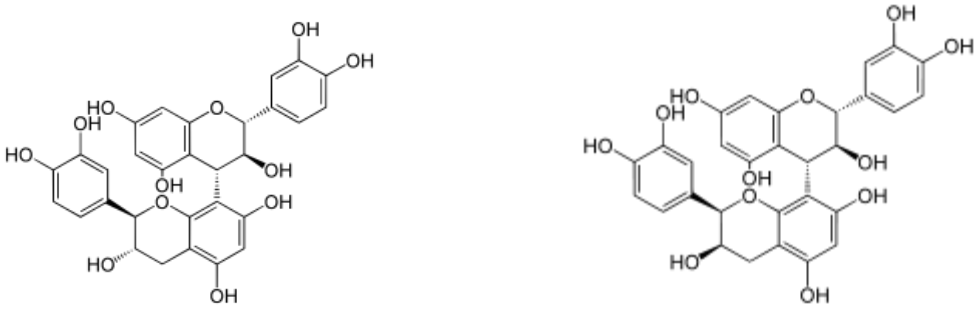

***Figure 17**.Procyanidin B3 (left) and Procyanidin B4 (right). Adapted from PubChem (pubchem.ncbi.nlm.nih.gov).*

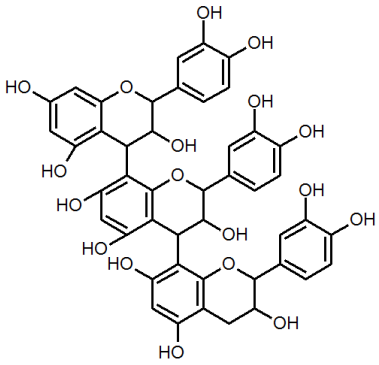

***Figure 18**. Procyanidin C. Adapted from PubChem (pubchem.ncbi.nlm.nih.gov).*

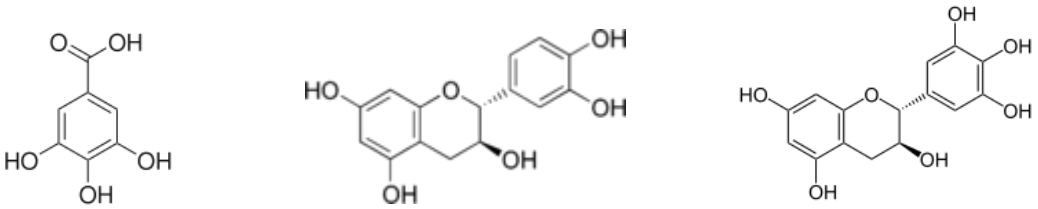

***Figure 19**. Gallic acid (left), Catechin (middle) and epigallocatechin (right). Adapted from PubChem (pubchem.ncbi.nlm.nih.gov).*

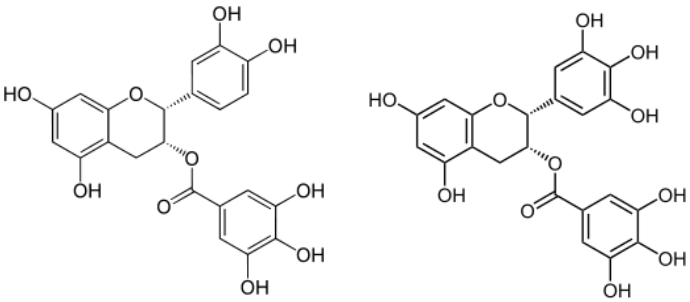

***Figure 20**. Epicatechin gallate and epigallocatechin gallate (right). Adapted from PubChem (pubchem.ncbi.nlm.nih.gov).*

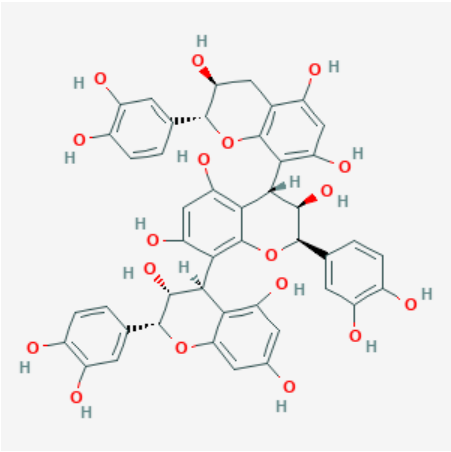

Figure 21. *Procyanidin T2. Adapted from PubChem (pubchem.ncbi.nlm.nih.gov).*

Procyanidins were analysed by injecting 3µl of wine samples through a Rapid Resolution Liquid Chromatography (RRLC) using a Zorbax Eclipse XDB-C18 (50 × 30; 1.8µm) followed by a RRLC in-line pre-column (4.6 mm, 0.2µm) at 30ºC. HPLC injection volume was 1.4µL, with a 0.7mL/min flux. Mobile phase A: water (0.1% formic acid), mobile phase B: methanol (0.1% formic acid). Column temperature: 30 ºC, Detector DAD: *Conditions TOF Dual Ionisation ESI:*_Temperature: 350 ºC, Flux drying gas (N2): 12 L/min, Nebulisator: 60psi, Polarity: Negative. *Analysator:* Mass range: 100-2000 m/z, Capillary Voltage: 3000V, Fragmentor: 150V, Skimmer: 60V, Octopole RF Peak: 250 V, Mass reference: 112,985587, 966,000725. Phenolic compounds were identified according to their order of elution, retention times of pure compounds (gallic acid, catechin, procyanidin dimer B2, mono gallate dimer, procyanidin trimer C1 and epicatechin gallate) and their molecular masses .

3.11 Experimental design

Experimental design was carried out during two seasons, 2009 and 2010 in 5 locations (Site 1, Site 2, Site 3, Site 4 and Site 5) of *cv*. Carignan and in 2010 and 2011 in two locations of *cv*. Grenache (Site 6 and Site 7) in the DOQ Priorat (Catalonia, Spain). The plots have different altitude, orientation and exposure (**Table 3**) located on hillsides and fluvial terraces, formed by the *Llicorella* (slaty schist soils). Soil water monitoring was performed with capacitance sensors (ECH_2O, Decagon), located at each vineyard (**Figure 7**). In all mesoclimatic locations variations were observed (climate, topography), characterized using agroclimatic stations (Decagon®). The below table shows the location, altitude, exposure, slope and planting distance for each vineyard.

***Table 3**. Summary of locations, Sites, variety, altitude, vineyard exposure, planting distance and slope.*

Vineyard Location	Town	UTM	Variety	Code	Altitude	Exposure	Planting distance	Slope
Site 1	El Molar	41°09'21"N 0°42'59"E	Carignan	EM, EMDA	220m	SW	2.10x1.30	23.9
Site 2	El Molar	41°09'19"N 0°42'40"E	Carignan	EM, EMBA	185m	SW	2.10x1.30	23.3
Site 3	Porrera	41°10'51"N 0°52'26"E	Carignan	PO, POMO	425m	SW	1.40x1.20	49.9
Site 4	Porrera	41°10'58,3"N 0°52'32,9" E	Carignan	PO, POME	425m	SE	1.40x1.00	48.3
Site 5	Porrera	41°10'58,1"N 0°52'32,6"E	Carignan	PO, PODA	495m	SW	1.20x1.20	54.3
Site 6	El Molar	41°9'21,10"N 0°43,4'08"E	Grenache	EM, EMGRE	210m	SW	2.50x1.20	15.3
Site 7	El Lloar	41°10'5,64"N 0°43'17,18"E	Grenache	LO, LLOGRE	240m	SW	2.50x1.20	17.9

Based on the previous experiences carried out in the DO Terra Alta and in order to determine what mesoclimatic variations are also established for the DOQ Priorat at the level of each plot specifically and to be able to relate them to the phenological and physiological differences that are going to be studied, it was installed in each plot a portable and automatic weather station (type EM50 DECAGON (Decagon Devices Inc., Pullman WA, USA, www.decagon.com) associated with a data logger. The agroclimatic stations (DECAGON) located in each plot recorded temperature (°C), relative humidity (%), radiation (W m^{-2}) and precipitation (mm) on an hourly basis, which allowed the calculation of the vapor pressure deficit (VPD, kPa), the thermal amplitude (AT , °C), growing degree days (GDD_{10}, °C) and potential evapotranspiration (ET0, l m^{-2}) according to Hargreaves.

The water content in the soil was measured by means of capacitance sensors (ECH2O, DECAGON) in a profile of up to 60 cm depth and expressed as a water layer (SWCWL, l · m^{-2}) then calculated as % of Volumetric Water Content (VWC). These data allowed the calculation of daily and seasonal climatic indices that are commonly used in different predictive models and in relation to the phenological parameters recorded. Among others, it was also calculated the average temperature of the growing season, the average maximum temperature of the growing season, the average minimum temperature of the growing season, the average temperature of the ripening period, the degree days of growth, using different base temperatures (Winkler et al., 1974; Huglin, 1978; Bind et al., 1996; Jones and Davis, 2000; García de Cortázar, 2006).

The determination of the total leaf area (TLA; m^2) was carried out at pea size (PS), veraison (V), ripeness (RP) and post-harvest (PH) (Carbonneau 1976; Cuevas 2001). In each of these phenological stages, at predawn (PD) and noon, the leaf water potential (LWP) was measured, using the Scholander pressure chamber (ARIMAD©). Also, the leaf temperature exposed to the sun were measured by an IR thermometer (Testo©). At the key moments

of the vegetative and productive cycle (pea-sized berry, veraison, full maturation (two or three weeks after veraison) and on the day of harvest), specific measurements were sampled providing information on the physiological state of the grapes, vigour and the level of stress. At these physiological stages, leaf samples were extracted to determine the concentration of phytohormones (abscisic acid) in the leaf to evaluate the levels of this hormone in order to calculate which is the moment of more severe water stress. This aimed to determine the variations in plant physiology between plots in the same area, potentially attributable to mesoclimatic variations. It was determined the mesoclimatic variations between plots to relate them with phenological or physiological variations of Grenache and Carignan.

As mentioned before, the wine-growing area of Priorat is located in the central part of the province of Tarragona, where vine cultivation is distributed at altitudes ranging from 100 m and 700 m above the sea level. The crop configuration is characterized by slopes that exceed 15% in most cases. The typical soil formed by schists allows rapid drainage that together with the hot summers and low rainfall characteristic of the region, configures an ecosystem in which drought prevails. The choice of the two locations for Carignan responds to the fact of the existence of two clearly differentiated mesoclimates in Priorat: the subzone with maritime influence in which the thermoregulatory breezes directly affect (Porrera) (**Figure 23**) and; the subzone at the other end, which opens towards the Ribera del Ebro, warmer that prevents the arrival of sea breezes (**Figure 22**) (El Molar).

Figure 22. *Location 1: NDVI Map for Site 1 (El Molar, EM, EMDA) and Site 2 (El Molar, EM, EMBA). Normalized difference vegetation index. Adapted from CESENS® (Encore Lab SL, Logroño, Spain)*

***Figure 23**. Location 2: NDVI Map for Site 3 (Porrera, PO, POMO), Site 4 (Porrera, PO, POME) and Site 5 (Porrera, PO, PODA). Normalized difference vegetation index. Adapted from CESENS® (Encore Lab SL, Logroño, Spain)*

Regarding Grenache, two vineyards are analysed here (**Figure 24**), both grafted onto R110. The plots are located in the townships of El Molar (EM) and El Lloar (LO), and studied during two distinctly different vintages: 2010 and 2011. Soils in both are typical of the region, characterized by poor, dry, and pebbly schist. The USDA classification for EM is sandy loam and silty loam for LO, both are of a co-alluvial origin formation. The terraces are naturally located at progressive topographic heights. Grenache vines in LO are 14 years old, and are growing in east-south facing terraces; EM vines are 16 years old and south-facing. Vine spacing is 1.2m and the inter-row distance is 2.5m. VSP trellising (70cm high) and bilateral cordon pruning characterize both vineyards. Grenache is distributed in terraces at different levels, conducted in trellises with a height of vegetation between 60-70 cm. Short pruning is performed on all vines and the trellises are conducted in a vertical system in a bilateral cordon. The soils are of colluvial origin, formed by a mixture of slate and calcareous materials, with loam to clay loam textures and with a percentage of stoniness that depends on the level gradient. Harvest dates in this location are traditionally mid-September. Three plot replications of each combination vigour/variety were randomly distributed in the vineyards, with each elementary plot consisting of 30 vines.

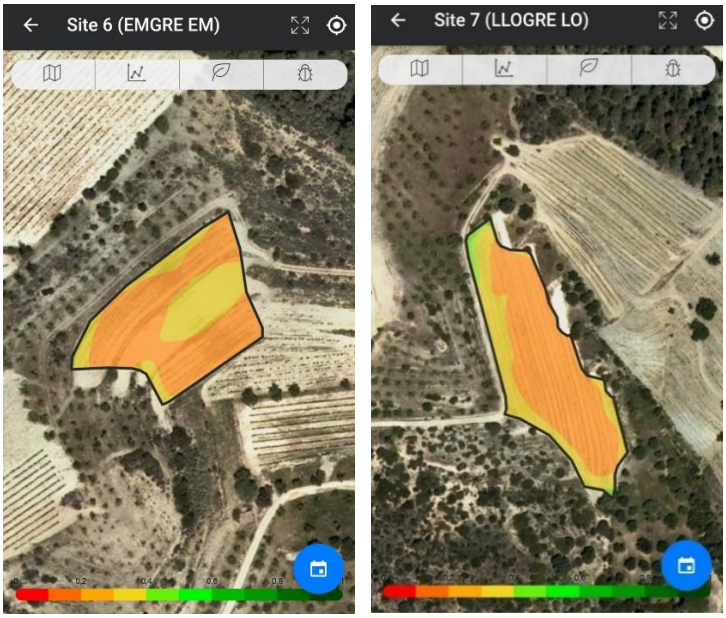

***Figure 24**. Location 3: NDVI Map for Site 6 (El Molar, EM, EMGRE) and Site 7 (El Lloar, LO, LLOGRE)*

Each of the vineyards will monitor the maturation process. Each terrace or hillside is divided into 3 blocks or elementary plots will carry out the different measurements and samplings in triplicate. 3 vines from each block are marked for non-destructive follow-ups (phenology). For the control and monitoring of the ripening of the grapes, samples of 450 berries were collected; 100 for the analysis of the basic parameters and the rest to proceed to determine the phenolic ripening of the berry.

The follow-up consisted of:

1- Berry growth (weekly): berry size evolution (weight and / or volume), from veraison to harvest.
2- Composition of the grape (weekly)
    2.1. Basic analysis of the must (brix by refractometry, TTA, pH, malic acid).
    2.2. Grape phenolic ripening analysis:
        a) Anthocyanins in skins (maceration in ethanol and HCl at pH 1 and reading at 535nm)
        b) In whole berry, in addition to determining the total polyphenol index (A_{280}), extractable and total anthocyanins according to the Glories methodology (Ribéreau-Gayon, 2000; Mateos, 2003), total tannins will also be analysed (Ribéreau-Gayon and Stonestreet , 1965, 1966), catechins by the DMAC method (Vivas et al., 1994) and the colouring intensity will be determined by measuring absorbances at 420, 520 and 620nm
        c) Determination of phenols in seeds after extraction in methanol: IPT, tannins and DMAC

d) Characterization of individual anthocyanins and procyanidins by HPLC. and also performing the detection at the lengths of 530, 420, 520, 620 and 280 nm (Hebrero et al., 1989).

3- Performance

3.1- Yield components at harvest date: kg / vine, bunches / vine, average bunch weight, berries / bunch.

3.2- Vegetative expression and vigour; pruning weight of vines during winter rest. Ravaz index.

3.3- Small-scale fermentations of each of the plots according to the red winemaking protocol. In wine, the alcoholic degree, total acidity, sulphur dioxide, pH and colour parameters will be determined: colouring intensity, total phenolic compounds (A_{280}), total anthocyanins, total tannins.

3.12 Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) to determine statistically different values at a significance level of $p \leq 0.05$. The *Tukey* test was applied to compare the 4 established treatments. All statistical analyses were performed using SPSS 17.0 program for Windows.

The water potential, leaf temperature, and grape and wine composition were evaluated through one-way ANOVA, and when $P<0.05$, Tukey post-hoc tests were used. A Pearson correlation matrix was calculated for all parameters with a significance level (α) of 0.05.

CART (classification and regression trees) analysis was performed using XLSTAT (Microsoft Excel statistical add-in). The decision tree method is a powerful and popular predictive machine learning technique that is used for both *classification* and *regression (Breiman et al., 1984)*. Thus, the methods are also known as Classification and Regression Trees (CART). The algorithm of decision tree models repeatedly partitions the data into multiple subspaces, so that the outcomes in each final subspace are as homogeneous as possible. Amongst all measured variables, the CART technique acts as a predictive model that shows the more significant variables to distinguish each final subspace. The tree models predict the outcome by asking a set of *if-else* questions. Regression tree analysis predicted the outcome as a real number (leaf temperature and water potential). The start of the tree was at the root node; for each variable, CART finds the set that minimizes the sum of the node impurities in the two child nodes and chooses the split that gives the minimum overall variable and set. The measure of the node impurity is based on the distribution of the observed values in the node; splitting stops if the relative decrease in impurity is below a pre-specified threshold.

The effect of tank size on wine composition was evaluated through one-way analysis of variance (ANOVA); $P < 0.05$, and the *Tukey post-hoc* test were used. The comparison of small-scale wines to commercial-sized tanks was performed using Principal Component Analysis (PCA), considering the 2500 L tank as a supplementary item; i.e. they were not included to calculate the principal components (PC), but to evaluate its performance. Statistical analyses were performed using R (R Core Team, 2014, Foundation for Statistical Computing, http://www.R-project.org/), and the FactoMineR and "factoextra" packages for personal computer (PC) calculation and graphical representation, respectively.

Chapter 4. Mesoclimatic Characterisation of Carignan and Grenache in the Priorat

4.1 Chapter summary

This chapter deepens on the characterisation of cultivars Carignan and Grenache in the selected plots. Additionally, the comparison of both grapes in two different vintages, helps to understand the physiology of each cultivar under water stress conditions, which are very common in DOQ Priorat. After understanding both varieties it is used a statistical tool to classify the vineyards, given the difficulty to assess water stress under several abiotic factors.

4.2 Mesoclimatic characterisation for cv. Carignan (El Molar- and Porrera-)

4.2.1 Climatology and soil

The climate of the DOQ is characterized by cold temperatures during winter and very high temperatures in summer. The vineyards located on hillsides and terraces are dry, however, the influence of the sea breeze, the *garbinada* wind, make the temperatures soften in summer, increase the relative humidity and decrease evaporation, and in most cases involves a delay of ripening. On contrast, the *serè* is a cold, dry wind that blows from the northwest along the Ebro Basin and comes more or less around the Priorat. In addition, the altitude of the plot is given by the location of towns between 110 m and 220 m, and between 200 and 700 for the higher altitude vineyards. The annual precipitation is between 450 and 500 mm, and rainfall are abundant between the end of October and November. Data that characterize climatic variation between small plots are essential for improving crop management under such extreme conditions.

The weather station (Agro-climatic network in Catalonia, XAC) provided supplemental data on the weather conditions in the study area. The climate in the Priorat region is characterized by high temperatures during the summer, drought, and steep poor stony soils and is thus highly vulnerable to climate change.

***Figure 25**. Installation of weather stations and soil sensors in Porrera. Old vines are planted in steep slopes of Llicorella.*

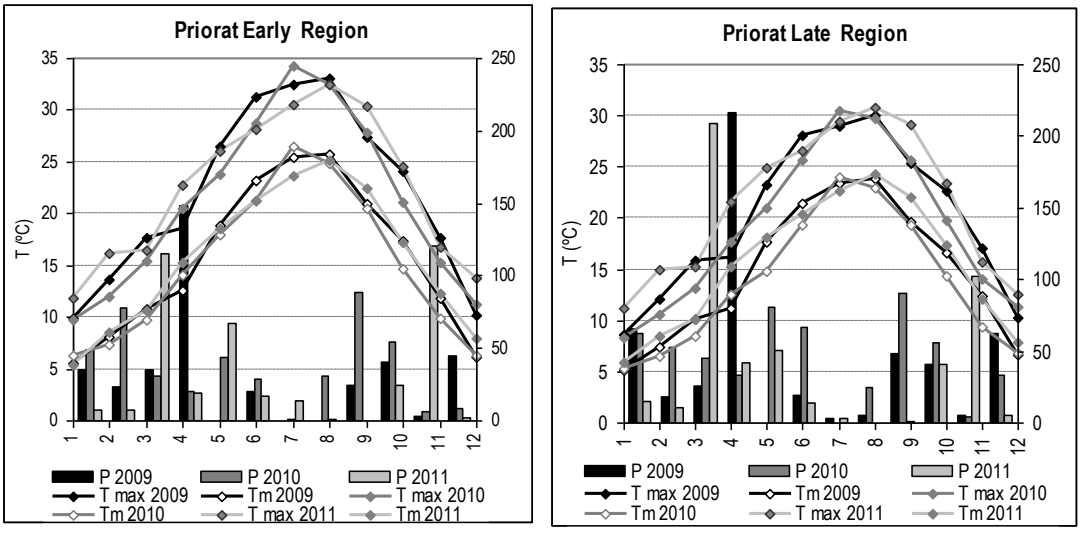

***Figure 26**. Annual climate diagram in early (EM) and late (PO) regions. Monthly Rainfall (P), monthly average Temperature (Tm), Maximum Temperature for 2009, 2010 and 2011.*

In 2009, in the early mesoclimate (El Molar, EM), the minimum temperature differences between Site 1 and Site 2 were 7°C, except in early March to mid-May and the first 3 weeks of July, where the minimum temperature differences were up to 3°C lower in Site 1. These differences, along with a slightly higher maximum temperature in Site 2, resulted in a higher thermal amplitude (AT) on the vineyard, especially from mid-May to early July and from veraison (V) to ripeness (RP) (August 15–September 21). Only moderate rain values were recorded in June (20 mm and 19 mm in EM and PO, respectively), indicating that the summer was dry. The average temperature during the summer months was high, reaching 23.2ºC in June, 25.5ºC in July, and 25.8ºC in August in EM and 21.4ºC, 23.4ºC, and 23.9ºC in PO in June, July, and August, respectively.

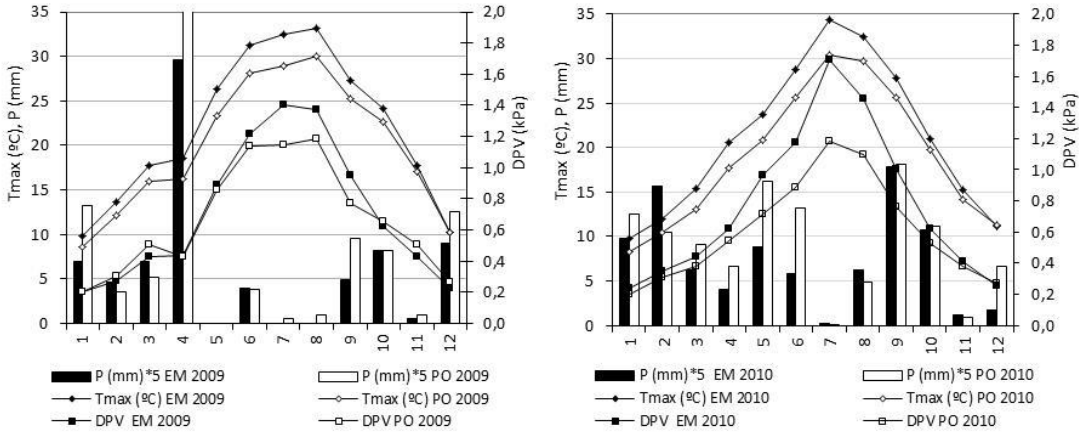

***Figure 27**. Annual climate diagram in early (EM) and late (PO) regions. Rainfall (P), Maximum Temperature (Tmax), and vapour pressure deficit (DPV) for 2009 and 2010.*

The 2009 vintage presents a very irregular distribution of rainfall in both locations. Rainfall was concentrated in the month of April, being scarce in the summer months. The last moderate rains were recorded in June, subsequently leading to a remarkably dry summer. The temperature and ETP in the Molar showed high values during June, July and August. In 2010, although the total annual rainfall does not differ significantly from 2009, the distribution of rain is more uniform throughout the year. Temperatures throughout the cycle were milder, although high temperatures were occasionally recorded in July (34.3°C in Molar and 30.4°C in Porrera). The vapor pressure deficit in 2009 follows the same trend as the maximum temperature where the highest values are reached in the months of June, July and August; 1.22kPa, 1.41kPa and 1.38kPa in the early region (EM) and 1.14kPa, 1.15kPa and 1.19kPa in the late region (PO). Thus, the extreme values are obtained in the early zone given that there is a higher atmospheric demand. In 2010, only in the month of July a remarkably high value of the VPD (1.8kPa) was observed. This value is only observed in the early zone, due to a specific climatic situation in which the temperature increased by

2ºC and the relative humidity decreased by 15%. The VPD in the late region (PO), on the other hand, presents VPD values similar to the previous year.

As expected, the late ripening region PO resulted in lower Temperature, VPD and GDD. Climatic data revealed 2009 as a warmer vintage. In 2009, temperature was very high especially in May and June, a fact that did not happen in 2010. The year 2009 showed a very uneven distribution of rainfall in both locations, EM and PO. Rainfall is concentrated in April representing 40% and 42% of the total of the year in EM and PO respectively, resulting in a very dry summer (**Figure 26**). In 2010, the two regions have followed the same trend with respect to temperature changes. Compared to the previous year there was very high Tm values only in July. T_{max} always reached higher EM values. Rainfall distribution was more uniform during the year. During the time this study was conducted, the maximum summer temperature difference between early and late regions ranged between 3 and 5 degrees (**Figure 27**). The lowest temperature recorded in the late region corresponds to a higher annual rainfall, which exceeds the early region at about 100mm. These results highlights the frequency of rain in 2010 (the year with the highest annual rainfall), which, together with a slight decrease in temperatures throughout the growing season, led us to characterize the vintage as warm. Although the years 2009 and 2011 are classified as dry, periods of higher temperatures in 2011 do not correspond to July, as expected. There was an unusually warm period in April and September, which formed a particular seasonal variability in 2011, most notably in the late region. The VDP, GDD_{10} and ET_0 are lower in the late region and in the temperate vintage.

Table 4. *Soil and Subsoil Texture.*

		< 2mm	>2mm	%	% silt	% clay	USDA-Classification
Site 1	Soil	49.6	50.4	56.7	22.3	20.0	Sandy clay loam
	Subsoil	42.3	57.7	57.0	21.3	20.7	Sandy clay loam
Site 2	Soil	22.9	77.1	53.3	26.7	20.0	Sandy clay loam
	Subsoil	13.4	86.6	50.7	25.3	24.0	Sandy clay loam
Site 3	Soil	40.1	59.9	50,3	29.7	20.0	Sandy clay loam
	Subsoil	37.5	62.5	28.0	67.0	5.0	Silty loam
Site 4	Soil	8.9	91.1	62.7	23.0	14.7	Sandy clay loam
	Subsoil	44.5	55.5	32.0	64.0	4.0	Silty loam
Site 5	Soil	29.2	70.8	74.0	16.0	10.0	Sandy clay loam
	Subsoil	19.5	80.5	30.0	65.0	5.0	Silty loam

Slate soils are stony, dry and poor. They are characterized by a high degree of porosity and good drainage as a result of the high percentage of burdensome elements they have, between 50 and 90% in particles of more than 2 mm in diameter. Site 1 shows a predominance of less rocky elements (>2mm) due to the flatter terrain of terraces situated on top of a soft ridge. Those soils accumulated the most amounts of sand and fine particles, while the gravel and stones were dragged down the slope and deposited in adjacent plots. In the late region, Site 3 is the plot that has an average percentage of finer elements, due

to the accumulation of eroded elements. Conversely, Site 2 and Site 5 contain a large proportion of gravel or coarse elements with higher drainage. Site 4 shows an important difference between the most stony topsoil and subsoil finely textured with good drainage.

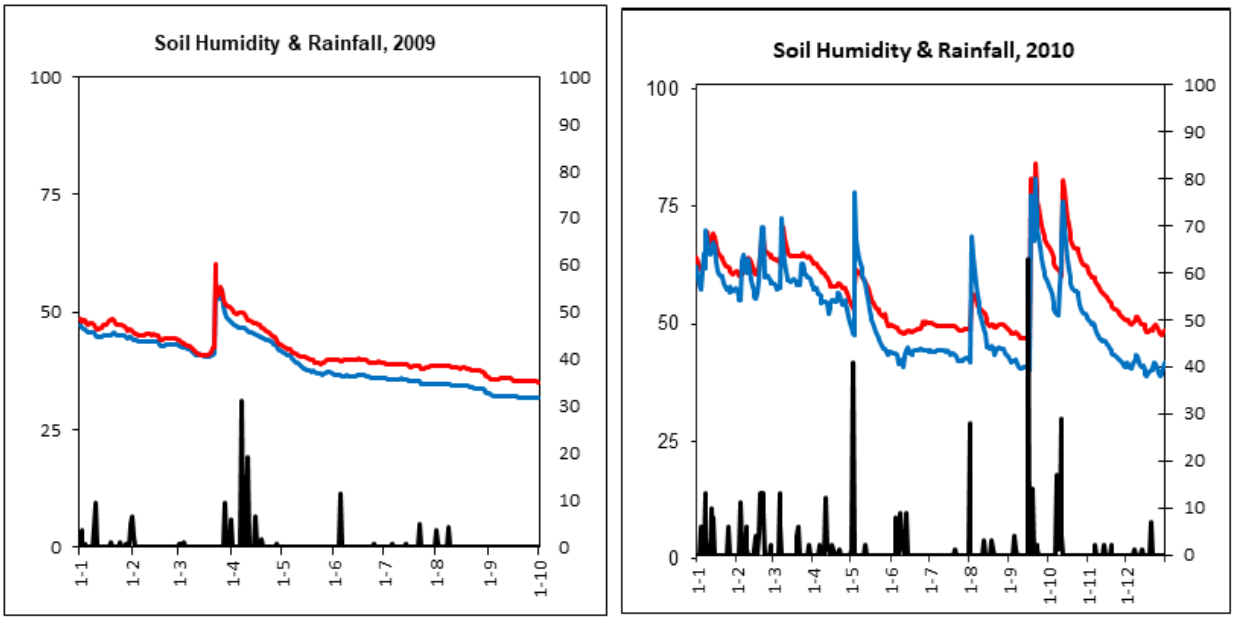

***Figure 28**. Rainfall (L/m²) (right axis) and soil humidity (L/m²) measured at 60cm (left axis). Site 1 (red) and Site 2 (dark blue). Rainfall (black)*

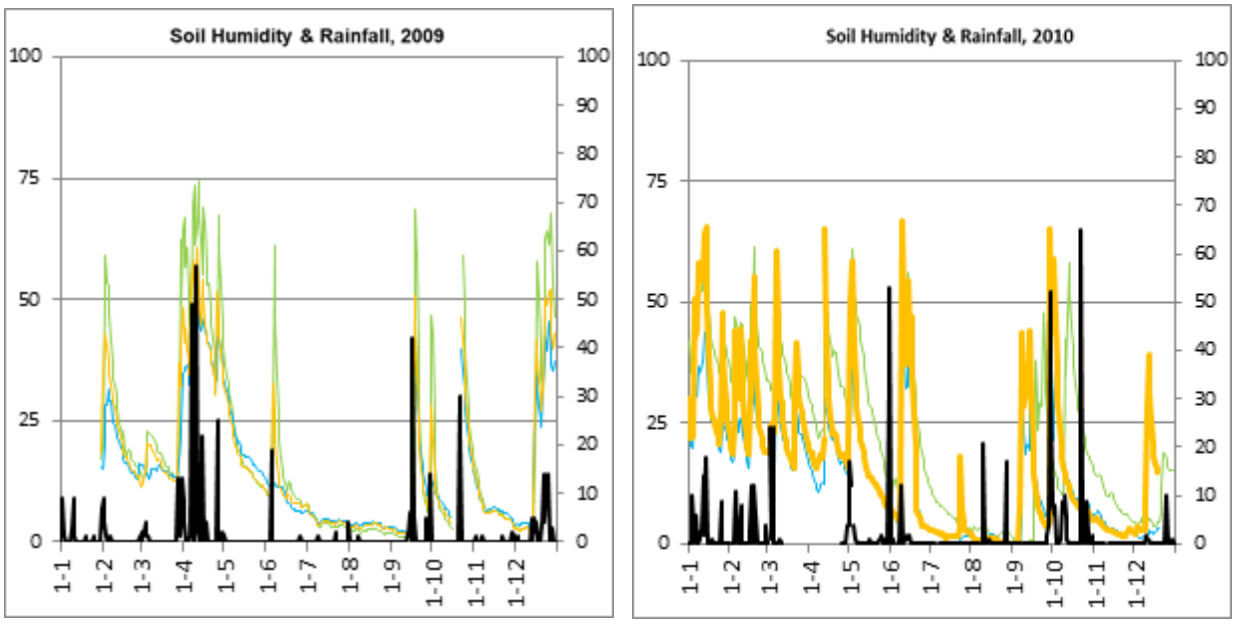

***Figure 29**. Rainfall (L/m²) (right axis) and soil humidity measured in % of VWC (Volumetric water content) measured at 45 cm. Sensor is located at 45 cm deep (left axis). Site 3 (orange), Site 4 (blue) and Site 5 (green)*

From the observation of **Figure 28** and **Figure 29** it is observed how after a rain event, the sensors respond and show a peak according to the amount of rain, indicative of the increase in the percentage of available water. In finer soils, the available water has a higher

basal line. It is also observed how the year 2010 presents a more distributed rainfall and with a less dry period during the summer months, when the plant has more water needs.

As shown in **Figure 30**, corresponding to the calculation of evapotranspiration, it is also observed that in a warm year (2009) the vineyards in the early ripening zone do not differ as much as in the late zone. On the other side, in Porrera plots, a different evapotranspiration is already shown between the vineyards in May-April. This suggests that late ripening mesoclimates, differentiate much more between plots than warmer areas. Plants suffer from water deficit during the summer period because temperatures are high and rainfall is virtually absent, as a result, evapotranspiration increases sharply with highs in July and August, which often causes a depletion of soil water reserves in El Molar. In addition, in stony slate soils, water reserves are depleted and water stress occurs. IN temeperate vintages, the stress would be partly alleviated by the precipitation of late spring and summer.

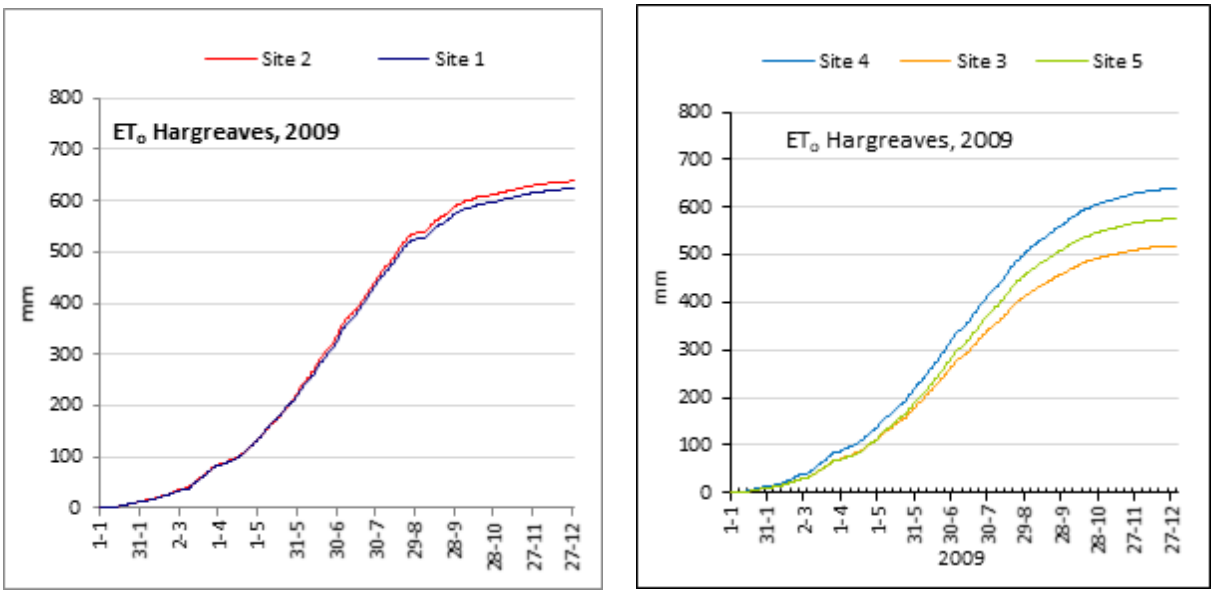

***Figure 30**. Evapotranspiration (ET_0). Site 1 (blue), Site 2 (red), Site 3 (orange), Site 4 (blue) and Site 5 (green).*

4.2.2 Phenology and vegetative growth

Phenological stages reflect among other things the environmental characteristics of the climate in the region where they occur. Consequently, long series of phenological observations may be used for the detection of climate variability or climate change. The effect of climate on phenology resulted in a greater variability of budbreak and veraison dates, depending on previous budbreak temperatures and those recorded in the spring. In the temperate vintage (2010) budbreak is delayed by 8 days in PO and by 11 days in EM when compared with the warm vintage (2009). However, in the temperate vintage, the differences are less notable at the beginning of budbreak and veraison between early and late regions (3 and 5 days respectively).

The high temperature in spring 2011 resulted in an earlier fruit set in the late region, which equilibrated the date recorded in the early region. Both regions accumulated approximately 150 degree days (GDD_{10}) compared to previous vintages, and involved a thermal integral to flowering of 585 and 536 GDD_{10} in the early and late region respectively. Moreover, the extended summer at the end of the ripening period in 2011 caused an advance of 15 days prior to the normal harvest date in the region. Most of the earlier studies on phenology and climate influence found a shortening of time between phenological stages, although most of them were conducted in cold weather. Date of harvest varied by 15 days in the warm year (2009), while the difference between regions was 10 days in the temperate years (2010) and by only a week in the warm year with seasonal temperature variability (2011). A delay in budbreak does not directly result in a delay of harvest, and it was observed that the warm years in late region brought an earlier date of harvest, particularly for 2011.

***Table 5**. Phenology dates of phenological stages in early (EM) and late (PO) regions.*

2009	**Bud break**	**Bloom**	**Fruit set**	**Pea size**	**Veraison**	**Harvest**	**Leaf drop**
EM	28-Mar	20-May	1-Jun	18-Jun	25-Jul	18-Sep	2-Nov
PO	3-Apr	26-May	10-Jun	25-Jun	4-Aug	4-Oct	31-Oct
2010							
EM	8-Apr	30-May	6-Jun	29-Jun	5-Aug	20-Sep	29-Oct
PO	11-Apr	5-Jun	13-Jun	6-Jul	11-Aug	30-Sep	2-Nov
2011							
EM	31-Mar	2-Jun	9-Jun	21-Jun	27-Jul	10-Sep	20-Nov
PO	3-Apr	3-Jun	9-Jun	27-Jun	5-Aug	15-Sep	5-Nov

***Table 6**. Growing degree days (accumulated GDD10) related to each phenological period in the early (EM) and late (PO) parcels.*

	Budburst	**Bloom**	**Fruit set**	**Pea size**	**Veraison**	**Harvest**	**Leaf fall**
2009							
EM	45	282	415	633	1180	2000	2133
PO	70,3	340	485	600	1230	1840	1960
2010							
EM	80	400	489	744	1348	2004	2220
PO	65,3	377	446	707	1222	1790	1950
2011							
EM	73	585	642	800	1313	2015	2584
PO	35,6	358	388	589	1029	1572	1857

***Table 7**. Length of different phenological periods from budburst in the early (EM) and late (PO) parcels.*

	Bud-Bloom	Bud-Fruit set	Bud-Pea size	Bud-Veraison	Bud-Harvest	Bud-leaf fall
2009						
EM	53	65	82	119	174	219
PO	53	68	83	123	184	211
2010						
EM	52	59	82	119	165	204
PO	54	62	85	121	171	204
2011						
EM	64	71	83	89	134	205
PO	61	67	85	93	134	185

These facts are associated with the high temperatures occurring in late August and even September in the Priorat, a situation in which the maturation of the grapes is accelerated. The total vegetative cycle from bud break to leaf drop shortened in the temperate year by 15 days in the early region and 7 days in the late region (2011 year). The long lasting summers evidenced the elongation of the cycle; leaf drop is delayed in the late region by 5 days and by 18 days in the early region. It is likely that the lowest temperatures occurring in October and the highest thermal amplitude (data not shown) accelerate leaf drop in late regions).

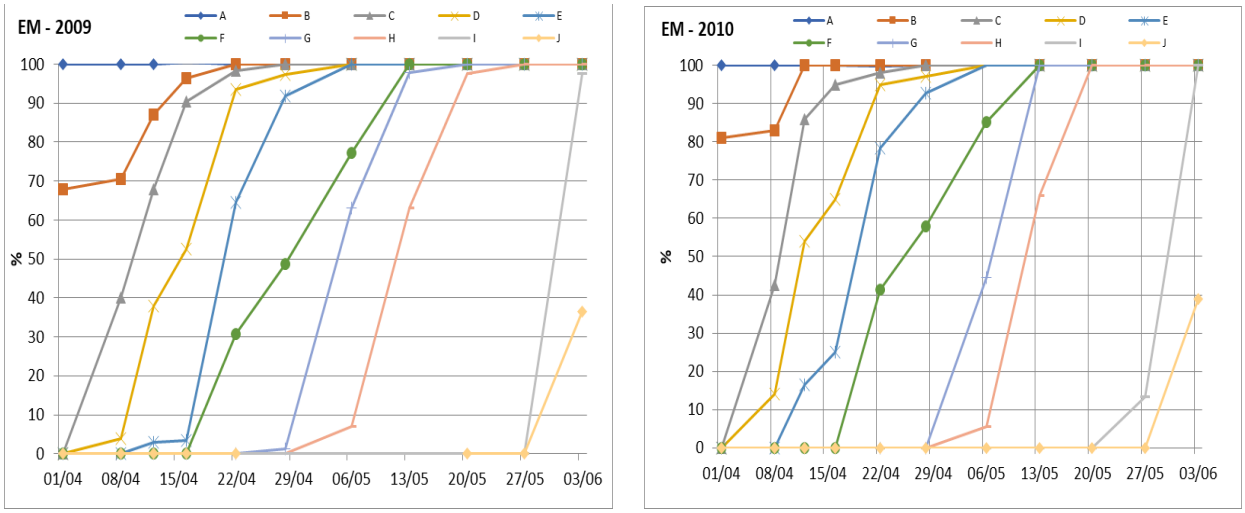

***Figure 31**. Evolution of the phenological stages of the Carignan variety in the towns of El Molar (EM) in 2009 and 2010. Percentage of Baggiolini phenological stages were measures from A to J.*

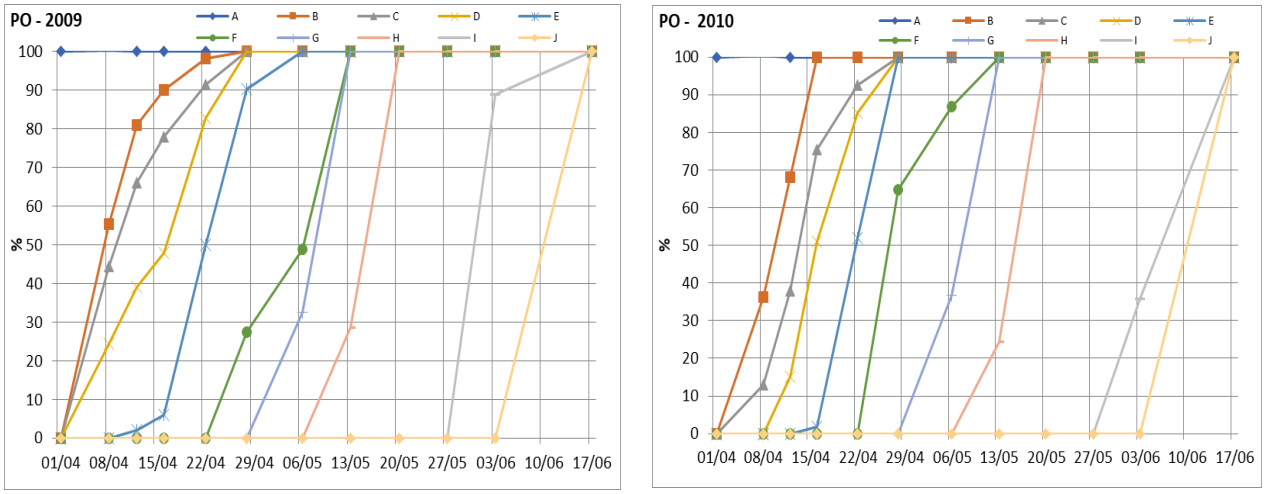

***Figure 32**. Evolution of the phenological stages of the Carignan variety in the town of Porrera (PO) in 2009 and 2010. Percentage of Baggiolini phenological stages were measures from A to J.*

Phenology is concerned with the periodic phenomenon of the vine growing cycle (bud burst, flowering, veraison), in relation to the climate. It is a veritable biological clock of the vines. The timing of the numerous operations in the vineyard (phytosanitary protection, defoliation, crop thinning, etc.) is undertaken in accordance with the phenological stages. Since the precociousness of the latter is directly linked to the temperature, the phenology is also a marker of global warming. The phenology of the vines responds very well to temperature. The vine growth cycle is more early-ripening in a warm year and more late-ripening in a cool year. It is also more early-ripening in a warm soil as compared to a cool soil. This monitoring of the phenology from the bud burst to the flowering and then the veraison provides knowledge, relatively early in the season, about whether the harvest will be earlier or later. This forecast becomes more precis over the successive stages.

During warm years the highest phenological differences between early and late regions were recorded, which reached the maximum of a week at budbreak and veraison. The start of budbreak is delayed in years of low winter temperatures, but this delay does not seem to affect the variations in the harvest date. To highlight the effect of seasonal climate variability: the temperature rise in spring and autumn affects a shortening between phenological stages in the late region, causing advances in flowering and harvest. The warm autumn also has a noticeable effect on the elongation cycle of the vine in the early region, prolonging the period from harvest to leaf drop.

Controlling the development of foliar mass as a way to improve the efficient use of water at the crop level has been a key factor. Given that soil analysis revealed Site 1 as a parcel having the finest texture and in Porrera the highest and steepest vineyard (Site 5) show the higher amount of gravels and stones; whilst Site 3 has a balance between fine elements and stones. Topsoil and subsoil layers are notably different in the Site 4 parcel. The two plots Site 5 and Site 1 are those with major differences between berry weights within the

two years of study, indicating a greater variability. Site 1 has the largest grape production, regardless of the vintage probably due to the fine soil texture. At the end of the maturation period, the ratio TLA / kg was higher in Site 1 and Site 5, corresponding with a greater leaf area in Site 1. In general the two plots are more irregular, with greater differences over the years with higher rainfall (2010) compared to dry years (2009), where the ratios do not show much difference. Concerning Ravaz-Index, the most unbalanced vigour corresponds to Site 1 (this explains major productions of grapes) and Site 4, which shows higher pruning weight, where the soil is more heterogeneous.

***Table 8**. ANOVA and test Tukey (p<0,05). Mean and SD for 2009 and 2010. TLA: total leaf area. Vegetative growth in the early (Site 1, Site 2) and late (Site 3, Site 4, Site 5) mesoclimates. [a]Values with different letters denote a statistically (p<0.05) significant difference. Mean and standard deviation.*

	Bunch weight (g)		Berry weight (g)		Kg/vine		TLA (m^2)		Ravaz Index		TLA/kg	
2009												
Site 1	131.0(72.0)	**a**	2.07(0.09)	**a**	1.21(0.48)	**a**	1.77(0.29)	**a**	2.4(0.4)	**c**	1.46(0.19)	**a**
Site 2	116.0(35.5)	**a**	1.82(0.04)	**b**	0.91(0.42)	**a**	1.21(0.31)	**b**	4.1(0.5)	**a**	1.33(0.34)	**a**
Site 3	134.0(20.5)	**a**	1.33(0.03)	**d**	0.85(0.10)	**a**	1.62(0.25)	**ab**	3.2(0.2)	**b**	1.79(0.21)	**a**
Site 4	148.0(12.8)	**a**	1.71(0.02)	**c**	0.96(0.09)	**a**	1.42(0.07)	**b**	4.3(0.1)	**a**	1.37(0.32)	**a**
Site 5	176.0(46.4)	**a**	1.02(0.07)	**e**	0.89(0.33)	**a**	1.76(0.31)	**a**	4.7(0.8)	**a**	1.89(0.34)	**a**
2010												
Site 1	127.5(39.1)	**a**	1.43(0.11)	**b**	1.30(0.60)	**a**	1.46(0.45)	**a**	5.6(2.0)	**a**	1.23(0.20)	**b**
Site 2	143.4(37.4)	**a**	1.51(0.08)	**b**	1.10(0.40)	**a**	1.62(0.43)	**a**	4.7(1.3)	**a**	1.50(0.10)	**ab**
Site 3	79.9(39.8)	**a**	1.62(0.10)	**ab**	0.90(0.40)	**a**	1.83(0.89)	**a**	3.3(1.0)	**a**	2.03(0.40)	**a**
Site 4	99.4(14.6)	**a**	1.40(0.09)	**b**	1.00(0.30)	**a**	1.88(0.26)	**a**	6.2(2.9)	**a**	1.86(0.50)	**ab**
Site 5	102.0(14.6)	**a**	1.71(0.03)	**a**	1.10(0.30)	**a**	1.75(0.60)	**a**	6.5(2.9)	**a**	1.59(0.50)	**ab**

The weight of the clusters and the production per vine do not show significant differences due to the great dispersion in the results, a consequence due to the great heterogeneity present between clusters of old vines. The berry weight, although it presents significant differences, shows a great oscillation of values in 2009, in a range between 1.02-2.07 while in temperate vintage the oscillations are smaller (1.40-1.71). In the plots examined in both vintages and viticultural regions (early and late) it is observed that the worst balanced Ravaz indices (3.2 and 3.3) and excessively high TLA/production ratios (1.79 and 2.03) they are in the Site 3 (POMO) vineyard. The conditions that exist in this vineyard favour a greater vegetative development that translates into greater vigour to the detriment of production (0.9 and 0.85).

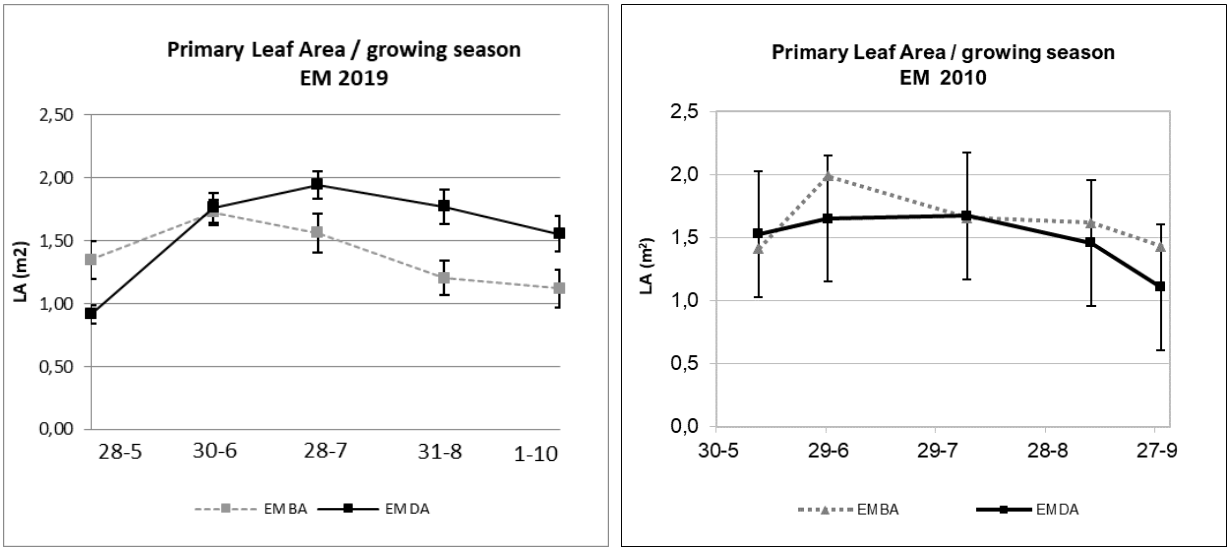

***Figure 33**. Primary leaf area during the growing season in El Molar for Carignan. Site 1 (black) and Site 2 (grey).*

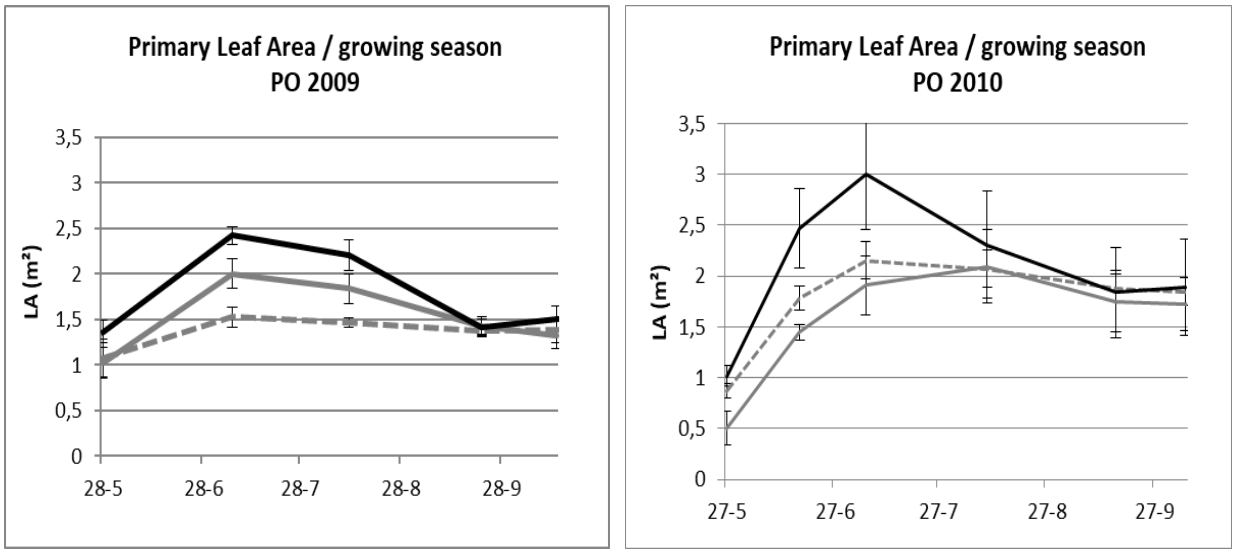

***Figure 34**. Primary leaf area during the growing season in Porrera for Carignan. Site 3 (black) and Site 4 (grey) and Site 5 (scattered).*

***Figure 35**. Carignan Site 1 El Molar, July 14th and Sep 7th 2009.*

***Figure 36**. Carignan Site 2 El Molar, July 7th and Sep 2nd 2009.*

***Figure 37**. Site 3, 4 and 5 Porrera, Sep 25th (left), Oct 15th (middle) and Oct 27th (right) 2009.*

Figure 35 shows an example of the plants sampled on plot Site 1 on July 14th, 2009 and the same plant sampled on September 7th, 2009. It is observed how the leaves in September are folded to prevent water loss and its appearance is less intense green. The installation of the soil humidity sensors was done in each plot. **Figure 36** shows the same effect in the Site 2 plot, in the photo on the left a greater greener colour is observed in the leaf, showing the Site 2 plot a greater vigour. **Figure 37** shows the 3 pictures of the same plot in the different stages of the phenological cycle on the Porrera estate, pictures were taken on September 25th, October 15th and October 27th, 2009.

4.2.3 Leaf water potential

The Carignan variety shows a clear decrease in the water potential in the ripening season, reaching values of -2 MPa in temperate year (2010) and early mesoclimate (El Molar); and in the warm (2009) vintage and late mesoclimate (Porrera). Carignan shows a recovery of the water potential in warm year and early mesoclimate; this is corroborated with early basal leaf yellowing and presence of raisins in the bunch, which does not occur in the other sites.

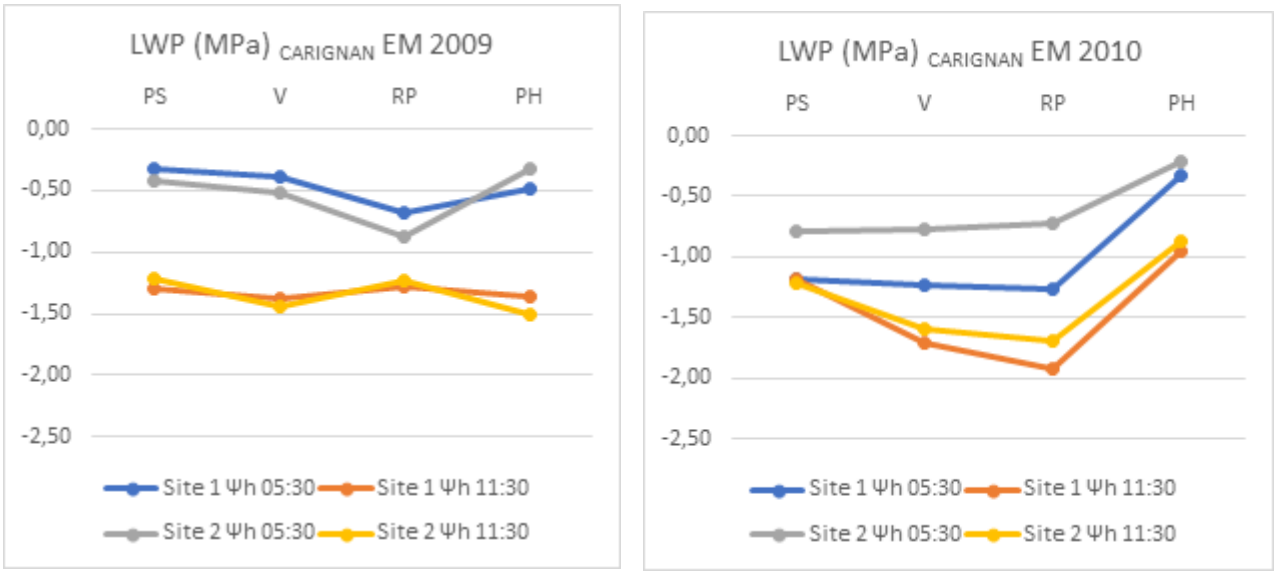

Figure 38. *EM (El Molar) Leaf water potential (LWP) during the growing period from PS (Pea Size) to PH (Post Harvest) during 2009 and 2010. Hours are shown in solar time.*

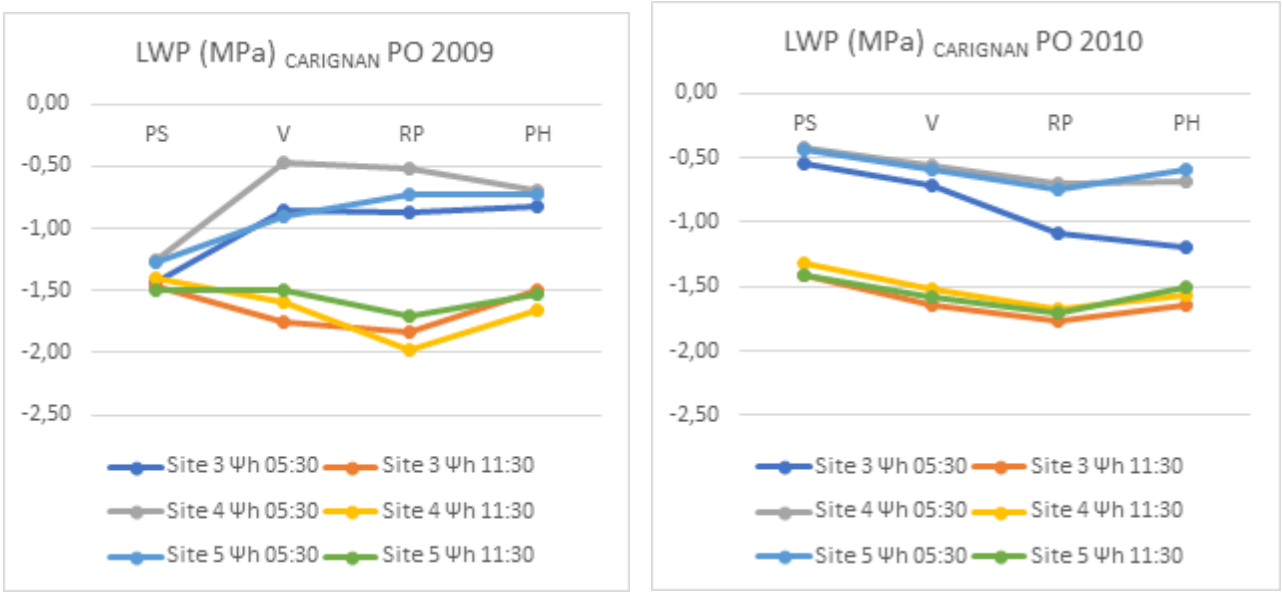

Figure 39. *PO (Porrera) Leaf water potential (LWP) during the growing period from PS (Pea Size) to PH (Post Harvest) during 2009 and 2010. Hours are shown in solar time.*

Even when soil water content diminishes, the Carignan leaf water potential (LWP) falls, and the plant suffers increasing stress. In general, grapevine is considered a water stress avoidant species, with a tight stomatal control. However, some varieties have shown a more efficient stomatal control than others. Our study revises, once more, the varietal behaviour of Carignan grapevine and tries to determine which behaviour confers best stress tolerance. Carignan would show a more anisohydric behaviour but under severe

drought conditions the vines result on a yellowing of the leaf and an increased number of raisins.

***Figure 40**. Unripe bunch of Site 1 Carignan in El Molar under severe water stress.*

4.2.4 Leaf temperature

The leaf temperature was measured between PS and PH at 8:00 AM and 12:00 PM. Temperature measured in the morning at 8:00 AM showed different tendency in Site 1 and 2 than Sites 3, 4 and 5. It is observed that the difference between the leaf temperature and the air temperature at noon is maintained in a greater range in the Porrera vineyard, indicating a greater transpiration in the vineyards of Site 1, 2 and 3.

Leaf temperatures measured in the morning show a temperature balance between air and leaf, while temperatures measured at noon show how air temperatures are much higher than leaf temperatures. In the 2010 vintage. In neither case, the two temperatures become equal and therefore the plant continues to cool its leaves thanks to continuous transpiration. This confirms the good hydric status of the vineyards studied in 2010 for both mesoclimates confirming the milder vintage.

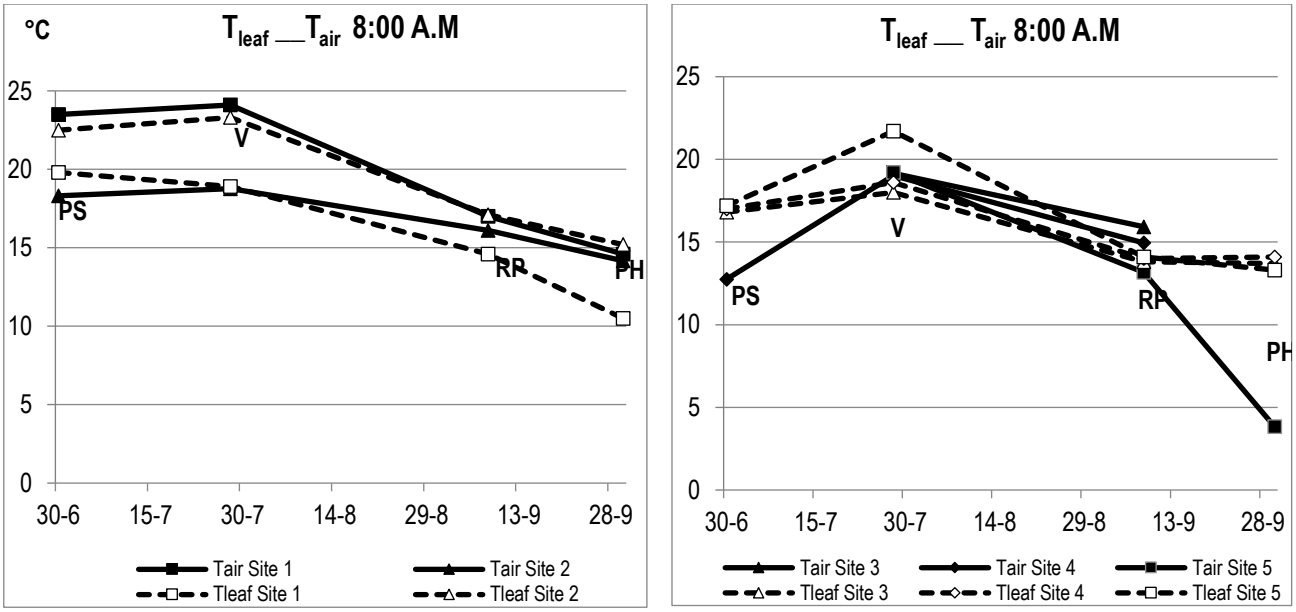

***Figure 41**. Measuremnets of T leaf and T air in El Molar during the vegetative cycle 2010.*

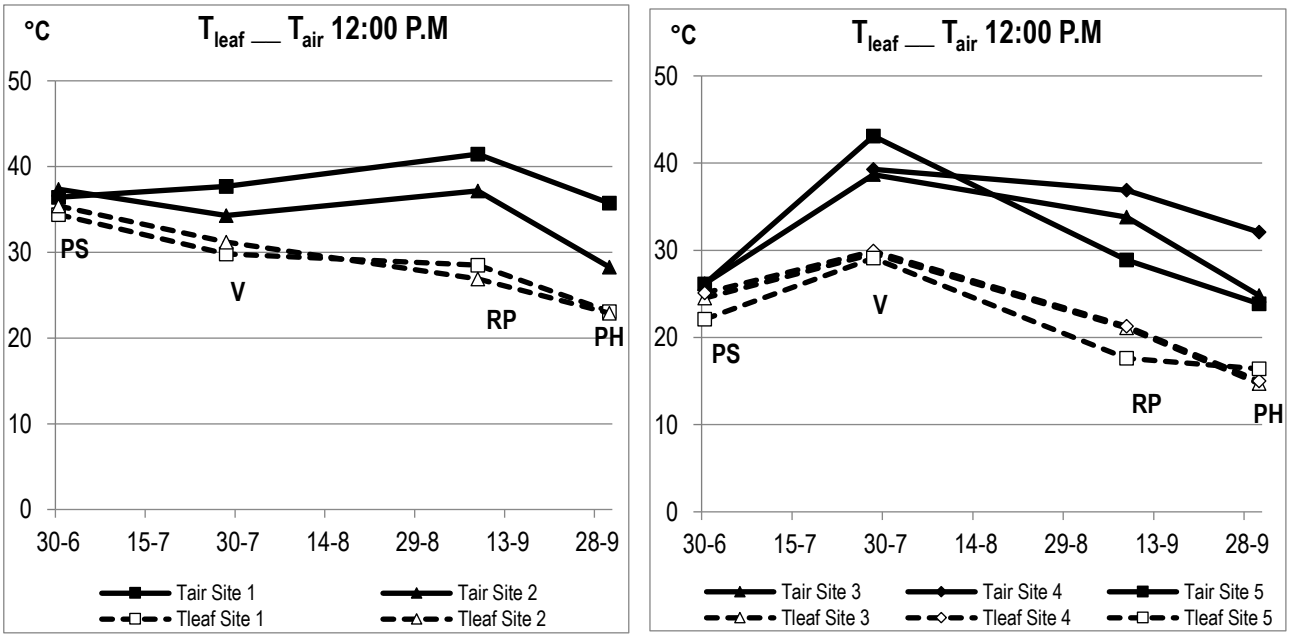

***Figure 42**. Measurements of T leaf and T air in Porrera during the vegetative cycle 2010.*

4.2.5 Stomatal conductance

Carignan in EM at Pea Size (PS) showed a higher transpiration at similar leaf water potential (LWP) values. As this cultivar shows early basal leaf yellowing and drop upon water stress, it could be ruled out the hypothesis of an increased transpiration due to a higher water availability during early stages of vegetative growth. On the contrary, during summer and the lack of water during veraison led us to confirm that decrease in stomatal conductance could be caused by a smaller loss of hydraulic conductivity due to low xylem vulnerability to cavitation. From our results the maximum value for g_s is seen at the beginning of the summer (PS) where El Molar Site 1 reaches values up to 500 mmol $m^{-2}s^{-1}$ and Porrera up to 300 mmol $m^{-2}s^{-1}$. This suggests that at PS there is no stomata closure for any of the plots as it shows higher values at noon. Site 1 and Site 2 show the highest differences between Sites compared to Porrera.

Stomatal conductance (g_s) decreases sharply under conditions of water stress as long as the season advances, resulting in an important limiting factor for photosynthesis around the vintage. In the period of PS the plants of Carignan show a stomatal conductivity at noon that is not altered by water stress. Instead from veraison and until harvest it is seen how the stomatal conductance at noon is lower, showing a more important stomatal closure. In the EM plots, in the warmer area, the stomatal conductance is very weak from the early hours of the morning. On the other hand, for the late mesoclimate in PO, it is seen how the conductance around the vintage and in the early hours of the morning still presents a certain stomatal opening while this decreases as we approach noon. This would indicate that the conductance is affected by the location of the plot.

The water available for the plant depends on the rainfall regime or the irrigation strategy used in the management of a vineyard, but it is not the case of study where no irrigation is available. It is known that under non-limiting conditions of soil water availability (g_s values greater than 150 mmol H_2O $m^{-2}s^{-1}$), the photosynthetic activity of the leaf remains in a range between 12 and 16 mmol CO_2 $m^{-2}s^{-1}$ (Escalona et al., 1999). The progressive advance of the hydric deficit during the summer, typical of the Mediterranean climate, determines a decrease in foliar photosynthetic activity that is reflected in a slowdown and subsequent stop of the vegetative growth, being able to substantially compromise the production and quality of the grape (Medrano et al., 2002; Chaves et al., 2010). This fact is accentuated with the more severe drought situation. In addition, under these growing conditions, grape production and quality can be compromised, which suggests the need for the use of irrigation systems that allow regulating the photosynthetic activity of the leaf and therefore growth, production and quality of the grape.

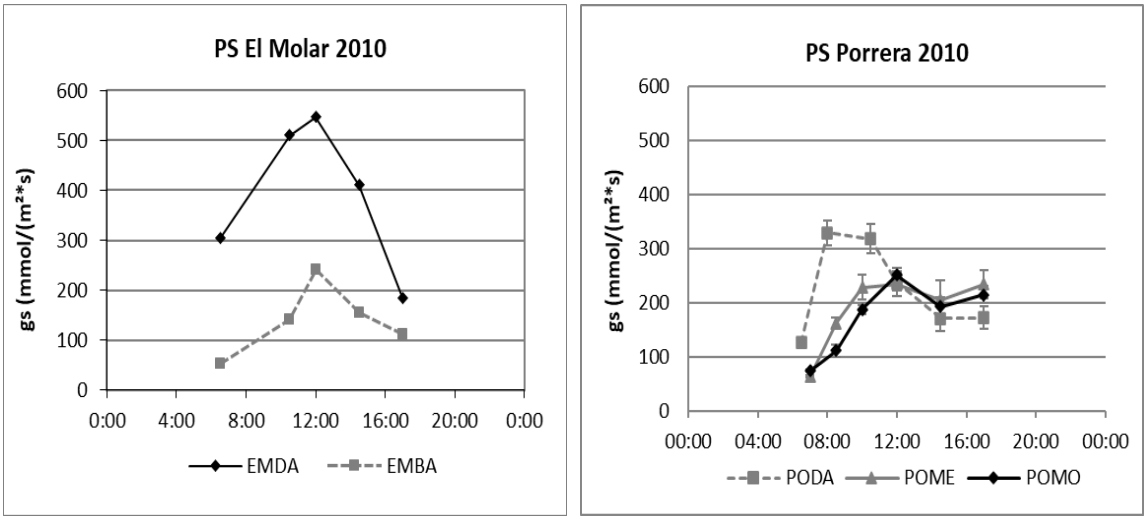

***Figure 43**. Stomatal conductance in 2010 at both location, El Molar (left) and Porrera (right). EMDA (Site 1), EMBA (Site 2), POMO (Site 3), POME (Site 4) and PODA (Site 5). Phenological stage: Pea size (PS).*

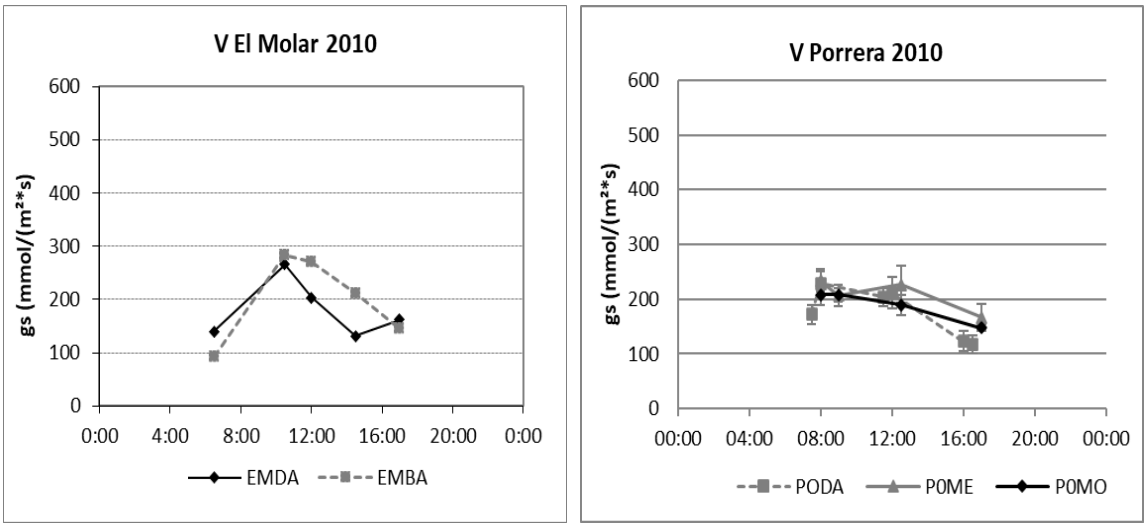

***Figure 44**. Stomatal conductance in 2010 at both location, El Molar (left) and Porrera (right). EMDA (Site 1), EMBA (Site 2), POMO (Site 3), POME (Site 4) and PODA (Site 5). Phenological stage: Veraison (V).*

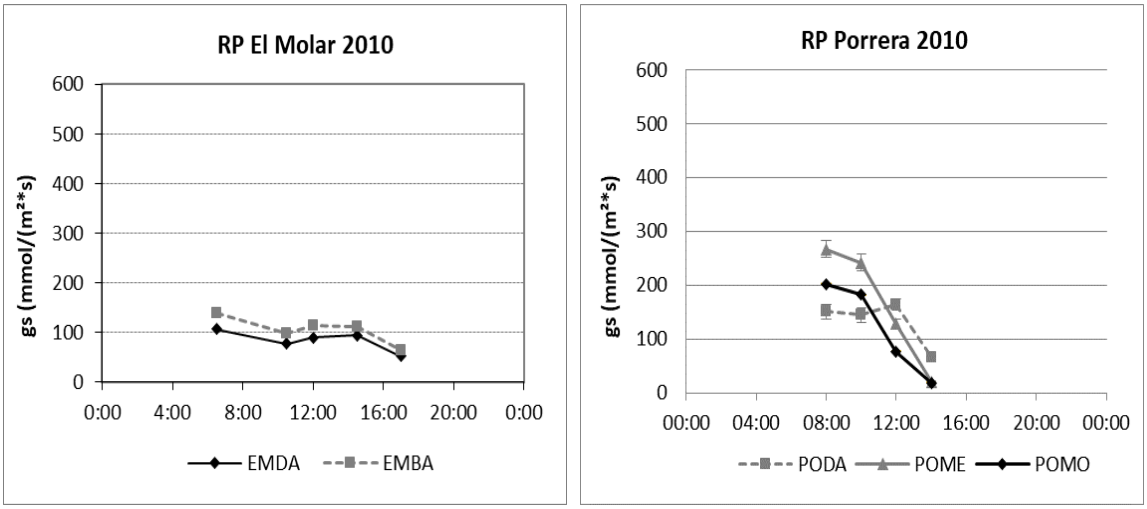

***Figure 45**. Stomatal conductance in 2010 at both location, El Molar (left) and Porrera (right). EMDA (Site 1), EMBA (Site 2), POMO (Site 3), POME (Site 4) and PODA (Site 5). Phenological stage: Ripeness (RP).*

4.2.6 Grape and wine Composition

Soluble solids (Brix) and total acidity are independent of vineyards and vintage (**Table 9**), Site 3 and Site 4 (west and east exposure) accumulated more Brix, with significant differences of almost 1 to 2.7 more degree Brix than other vineyards (2010). In 2009 differences are not as noticeable as 2010. As for the content of anthocyanins, an increasing trend associated with the late region in warmer years and in higher altitude vineyards was observed. Variations in the composition of the grapes are not attributable to berry weight, which showed no consistent differences between plots and years (data not shown)

Concerning the phenolic composition of the grape, only in warm years are significant differences observed between the study plots. The highest concentration in total and extractable anthocyanins correspond to the plots of the later region (PO), with maximum values reaching 1728 mg/L in Site 3. On the contrary, in the early region (EM) lower concentrations of anthocyanins accumulate (992 and 1116 mg/L). This results suggest that in a very vintage (2009) anthocyanins might degrade compared to the late ripening area (Porrera) where anthocyanins accumulate much more. In the temperate year 2010, the anthocyanin concentration did not vary between plots or between mesoclimates.

***Table 9**. Result of the composition of the grape in the maturation. ANT T: Total anthocyanins; ANT E = Extractable anthocyanins. TTA: Titratable Acidity. Statistical analysis ANOVA and Tukey's test (p <0.05) Average and standard deviation.*

	ANT T(mg/L)		ANT E(mg/L)		Brix		TTA(g/L)	
2009								
Site 1	1116(34)	**c**	531(82)	**c**	24.2(0.1)	**b**	5.08(0.01)	**a**
Site 2	992(46)	**d**	544(40)	**c**	23.7(0.1)	**d**	4.79(0.04)	**b**
Site 3	1728(153)	**a**	1000(27)	**a**	24.3(0.1)	**b**	3.89(0.07)	**d**
Site 4	1292(32)	**b**	756(66)	**b**	24.7(0.1)	**a**	3.42(0.05)	**e**
Site 5	1570(106)	**a**	997(17)	**a**	24.0(0.1)	**c**	4.03(0.05)	**c**
2010								
Site 1	1278(119)	**a**	699(55)	**b**	24.4(0.3)	**b**	4.64(0.14)	**a**
Site 2	918(59)	**b**	559(34)	**c**	23.5(0.3)	**c**	4.28(0.04)	**a**
Site 3	995(56)	**b**	713(25)	**b**	26.2(0.5)	**a**	3.82(0.15)	**b**
Site 4	1185(106)	**a**	760(25)	**b**	25.2(0.4)	**a**	4.48(0.08)	**a**
Site 5	1286(50)	**a**	867(28)	**a**	23.8(0.1)	**c**	4.53(0.09)	**a**

Analysis of the wines: The difference between the minimum and maximum values of alcoholic degree was 1.5 (range between 16.0-14.5% (v / v)) in 2009 and 1.1 (in range of 15.2-14, 2% (v / v)) in 2010. Regarding the TPI index, differences were found between plots of up to 23 units in 2009, while there were only 5 TPI units in 2010. The same happens in relation to tannins, observing differences of 1.5 (range between 2.9-1.4 g / l) for 2009 and

1.1 (range of 2.2-1.1 g/l) in 2010. On the other hand, the differences in anthocyanins Total comparing both years is less remarkable (between 30- 80 g/l).

The TPI index is around 45-50.8 in temperate years and 45-72 in drier and warmer years. Regardless of the year and the plots, anthocyanins are in a range between 441.3-525.8 mg/L. In warm years, grapes in the early zone reach a higher alcoholic degree than in the late zone, although there are no differences in temperate years between zones. However, the total acidity is higher in the late zone in both vintages. The results in TPI, pH and anthocyanins show variability between plots and vintages, however the concentration of tannins was always higher in wines from the early zone. In the warmest years (2009), wines acquire a greater alcoholic degree, acidity and lower pH in Site 1and Site 5. In the other hand, the results in the temperate year (2010) show more variability depending on the type of soil and slope of the plot. Wine phenolic composition does not seem to follow a behaviour strictly related to soil type of each plot, with a major importance of the interannual climatic conditions.

***Table 10**. Interparcel analysis of phenolic compounds in wines in 2009 and 2010. ANOVA and Fisher's test ($p < 0.05$) to reveal the differences between treatments. Average and standard deviation. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

	ABV		TTA(g/L)		pH		ANT T(mg/L)		Tannins (g/L)		TPI (A280)	
2009												
Site 1	16.0(0.4)	**a**	6.63(0.17)	**c**	3.17(0.09)	**bc**	398(10)	**c**	2.9(0.2)	**a**	72.4(3.2)	**a**
Site 2	15.2(0.6)	**abd**	6.40(0.15)	**c**	3.27(0.03)	**b**	467(4)	**a**	2.4(0.2)	**b**	66.6(6.2)	**a**
Site 3	14.8(0.1)	**bc**	6.39(0.03)	**c**	3.28(0.01)	**b**	471(22)	**ab**	2.2(0.3)	**b**	49.1(2.2)	**b**
Site 4	14.5(0.2)	**cb**	6.85(0.06)	**b**	3.35(0.01)	**a**	433(23)	**b**	1.7(0.1)	**c**	45.0(1.3)	**c**
Site 5	15.4(0.1)	**d**	7.62(0.07)	**a**	3.21(0.02)	**c**	452(6)	**b**	1.4(0.1)	**d**	45.6(0.6)	**c**
2010												
Site 1	14.5(0.1)	**b**	6.98(0.08)	**c**	3.22(0.02)	**c**	493(17)	**b**	1.9(0.1)	**b**	47.6(2.3)	**bcd**
Site 2	14.4(0.1)	**bc**	6.13(0.10)	**d**	3.52(0.01)	**a**	526(14)	**a**	2.2(0.1)	**a**	49.9(0.7)	**ab**
Site 3	15.2(0.2)	**a**	6.97(0.05)	**c**	3.54(0.00)	**a**	441(19)	**c**	1.1(0.3)	**d**	50.8(0.4)	**a**
Site 4	15.3(0.1)	**a**	7.45(0.07)	**a**	3.43(0.04)	**b**	456(18)	**c**	1.6(0.0)	**c**	48.4(0.1)	**c**
Site 5	14.2(0.2)	**c**	7.19(0.08)	**b**	3.44(0.00)	**b**	447(10)	**c**	1.1(0.2)	**d**	45.7(2.4)	**d**

4.2.7 HPLC-DAD-MS polyphenolic characterisation of Vitis vinifera L. cv. Carignan grape and wine

Carignan wines showed the highest concentrations of total anthocyanins in the dry and warm vintage. However, the nature of the anthocyanins led to variability in the extraction ratios (data not shown) and, as a consequence, the anthocyanin composition of the wines varied with respect to the grapes. In that respect, De Villiers *et al.* (2004) found that non-acylated glucosides were more easily extracted, followed by acetyl glucosides and, finally, p-coumaroyl glucoside, which are the most difficult to extract from grapes to wine. In this

study, the non-acylated compounds were also found at higher concentrations than their acylated counterparts. Site 1 in 2009 extracts 251.1mg/L being the lowest; site 2, 3, 4 and5 reach a higher concentration of anthocyanins up to 319.1mg/L. Instead in 2010 Site 2 extracts the highest concentration, 214.8mg/L being the other sites no lower than 187.5mg/L.

Table 11. *Anthocyanin composition of the wines issued from Site 1 and 2 (Molar) and Site 3, 4 and 5 (Porrera). 2009.*
[a]Values with different letters denote a statistically (p<0.05) significant difference. Mean and standard deviation.

Location	Site 1		Site 2		Site 3		Site 4		Site 5	
Sample	**wine (mg/L) Vintage 2009**									
Mv3G	164,1 ± 4,8	**a**	217,0 ± 4,5	**d**	222,6 ± 6,3	**cd**	203,6 ± 7,4	**b**	226,8 ± 1,8	**c**
Pt3G	7,6 ± 0,4	**a**	8,8 ± 2,3	**b**	7,7 ± 1,9	**a**	5,5 ± 1,8	**a**	6,9 ± 2,1	**a**
Dp3G	2,0 ± 1,7	**a**	2,7 ± 1,0	**b**	2,2 ± 1,8	**a**	2,0 ± 1,3	**a**	2,6 ± 0,6	**a**
Pn3G	4,6 ± 0,2	**b**	5,2 ± 0,6	**d**	5,5 ± 0,1	**c**	3,5 ± 0,0	**a**	4,7 ± 0,5	**c**
Cy3G	0,3 ± 0,0	**b**	0,3 ± 0,1	**ab**	0,3 ± 0,0	**b**	0,2 ± 0,0	**a**	0,3 ± 0,0	**b**
Total G	178,7 ± 6,8	***b***	234,0 ± 3,2	***ab***	238,3 ± 5,6	***a***	214,8 ± 10,3	***c***	241,4 ± 4,2	***a***
Mv3AG	32,2 ± 1,6	**a**	57,1 ± 1,9	**c**	54,8 ± 0,6	**b**	59,5 ± 0,5	**c**	64,6 ± 1,5	**d**
Pt3AG	0,8 ± 0,0	**a**	1,1 ± 0,0	**d**	1,0 ± 0,0	**c**	0,9 ± 0,0	**b**	0,9 ± 0,0	**b**
Dp3AG	0,3 ± 0,0	**a**	0,4 ± 0,0	**b**	0,3 ± 0,0	**a**	0,3 ± 0,0	**a**	0,3 ± 0,0	**a**
Pn3AG	1,5 ± 0,0	**a**	1,8 ± 0,0	**b**	1,9 ± 0,0	**c**	1,5 ± 0,1	**a**	2,0 ± 0,1	**d**
Cy3AG	0,2 ± 0,0	**ns**	0,2 ± 0,0	**ns**	0,2 ± 0,0	**ns**	0,2 ± 0,0	**ns**	0,2 ± 0,0	**ns**
Total AG	*35,0 ± 1,6*	***d***	*60,6 ± 1,8*	***ab***	*58,2 ± 0,6*	***a***	*62,4 ± 0,5*	***b***	*68,0 ± 1,5*	***c***
Mv3CG	28,7 ± 2,4	**a**	38,5 ± 5,6	**b**	41,3 ± 1,2	**b**	34,6 ± 7,9	**ab**	37,3 ± 7,6	**ab**
Pt3CG	3,9 ± 0,2	**a**	5,8 ± 0,0	**c**	4,4 ± 0,2	**b**	3,7 ± 0,2	**a**	4,2 ± 0,1	**b**
Dp3CG	1,3 ± 0,1	**b**	1,8 ± 0,0	**c**	1,2 ± 0,0	**b**	1,0 ± 0,1	**a**	1,0 ± 0,0	**a**
Pn3CG	2,5 ± 0,3	**ns**	3,0 ± 0,4	**ns**	2,8 ± 0,1	**ns**	2,0 ± 0,2	**ns**	2,8 ± 0,1	**ns**
Cy3CG	1,1 ± 0,0	**c**	1,2 ± 0,0	**d**	1,0 ± 0,0	**c**	0,7 ± 0,0	**a**	0,9 ± 0,0	**b**
Total CG	*37,5 ± 2,8*	***b***	*50,4 ± 6,0*	***a***	*51,0 ± 1,4*	***a***	*42,0 ± 8,2*	***ab***	*46,2 ± 7,7*	***ab***
G+AG+CG	251,1 ± 0,5	**c**	345,0 ± 7,1	**ab**	347,6 ± 6,8	**a**	319,1 ± 16,4	**b**	355,5 ± 6,6	**a**

As a result, it is observed that in warm mesoclimate and in warm year, the concentration range oscillates between 251.1 and 345.0 mg / L; in late mesoclimate and warm year the range oscillates between 319.1 and 355.5 mg / L. On the other hand, in a warm vintage, the concentration ranges are very different; in warm mesoclimate and temperate year the ranges are between 187.5 and 214.8 mg / L and in late mesoclimate and temperate year the values are between 197.6 and 202.8mg / L.

***Table 12**. Anthocyanin composition of the wines issued from Site 1 and 2 (Molar) and Site 3, 4 and 5 (Porrera). 2010.*
[a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.

Location	Site 1		Site 2		Site 3		Site 4		Site 5	
Sample	**wine (mg/L) Vintage 2010**									
Mv3G	134,7 ± 3,0	**b**	145,7 ± 2,3	**ab**	132,7 ± 2,8	**b**	136,3 ± 4,8	**b**	137,8 ± 4,5	**b**
Pt3G	2,7 ± 0,1	**b**	3,8 ± 0,5	**ab**	2,9 ± 0,2	**b**	3,6 ± 0,9	**a**	2,6 ± 0,3	**ab**
Dp3G	0,6 ± 0,9	**ns**	0,9 ± 0,1	**ns**	0,6 ± 0,6	**ns**	0,9 ± 0,4	**ns**	0,4 ± 0,1	**ns**
Pn3G	0,8 ± 0,1	**ns**	0,8 ± 0,1	**ns**	0,7 ± 0,1	**ns**	0,8 ± 0,3	**ns**	0,6 ± 0,1	**ns**
Cy3G	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**
Total G	138,7 ± 4,2	**b**	151,2 ± 3,0	**a**	136,9 ± 3,6	**b**	141,6 ± 6,3	**b**	141,4 ± 4,9	**b**
Mv3AG	25,7 ± 0,5	**c**	38,7 ± 1,2	**a**	35,3 ± 0,1	**b**	35,9 ± 0,0	**b**	36,4 ± 1,2	**a**
Pt3AG	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**
Dp3AG	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**
Pn3AG	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**
Cy3AG	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,0	**ns**
Total AG	*25,8 ± 0,6*	***d***	*38,9 ± 1,2*	***a***	*35,4 ± 0,1*	***c***	*36,1 ± 0,0*	***b***	*36,5 ± 1,2*	***abc***
Mv3CG	22,3 ± 0,3	**c**	23,6 ± 0,3	**b**	24,6 ± 0,5	**a**	24,1 ± 1,2	**a**	23,4 ± 0,7	**ab**
Pt3CG	0,4 ± 0,1	**b**	0,8 ± 0,1	**a**	0,5 ± 0,1	**b**	0,6 ± 0,2	**b**	0,4 ± 0,1	**b**
Dp3CG	0,1 ± 0,0	**b**	0,2 ± 0,0	**a**	0,1 ± 0,0	**b**	0,1 ± 0,1	**ab**	0,1 ± 0,0	**b**
Pn3CG	0,1 ± 0,0	**ns**	0,2 ± 0,0	**ns**	0,2 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**
Cy3CG	0,0 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,0 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,0 ± 0,0	**ns**
Total CG	*23,0 ± 0,4*	***b***	*24,8 ± 0,4*	***a***	*25,3 ± 0,7*	***a***	*25,1 ± 1,6*	***a***	*24,1 ± 0,8*	***ab***
G+AG+CG	187,5 ± 5,1	**c**	214,8 ± 4,6	**a**	197,6 ± 4,4	**b**	202,8 ± 7,9	**b**	202,0 ± 6,9	**b**

It is important to note that in reference to the composition of anthocyanins, there is an important relationship with the vintage and the situation of the plot in warm years and in temperate years it only has a direct relationship with the climatology of vintage, but not the location of the plot.

***Table 13**. Procyanidin composition of the wines issued from Site 1 and 2 (Molar) and Site 3, 4 and 5 (Porrera). 2009.*
[a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.

Location	Site 1				Site 2				Site 3				Site 4				Site 5			
Sample	**wine (mg/L) Vintage 2009**																			
Gallic acid	22,1	±	0,3	**a**	16,1	±	0,6	**b**	14,6	±	0,5	**bc**	15,3	±	0,5	**b**	13,9	±	0,2	**c**
Cat	10,7	±	0,2	**a**	7,7	±	0,1	**b**	5,0	±	0,1	**d**	5,4	±	0,1	**c**	4,7	±	0,1	**e**
EC	3,2	±	0,1	**a**	1,8	±	0,1	**d**	2,2	±	0,0	**c**	3,1	±	0,0	**a**	2,6	±	0,0	**b**
ECG	0,1	±	0,1	**ns**	0,1	±	0,0	**ns**	0,1	±	0,0	**ns**	0,1	±	0,0	**ns**	0,0	±	0,0	**ns**
Total Monomers	*36,1*	±	*2,5*	***a***	*25,7*	±	*1,8*	***b***	*21,8*	±	*3,7*	***bc***	*23,8*	±	*4,6*	***b***	*21,1*	±	*3,3*	***b***
pdB1	23,7	±	2,2	**a**	18,9	±	3,4	**a**	13,7	±	2,3	**b**	14,5	±	1,5	**b**	12,6	±	2,2	**b**
pdB2	8,6	±	0,9	**ab**	6,3	±	0,7	**c**	6,9	±	0,7	**bc**	9,6	±	0,8	**a**	7,1	±	0,6	**b**
pdB3	3,5	±	0,2	**a**	2,4	±	0,1	**c**	2,3	±	0,2	**c**	2,7	±	0,1	**b**	2,0	±	0,1	**d**
pdB4	8,5	±	0,9	**ab**	6,3	±	0,7	**c**	6,9	±	0,7	**c**	9,5	±	0,8	**a**	7,1	±	0,7	**bc**
pdB2MG1	1,7	±	0,1	**c**	2,5	±	0,1	**b**	3,0	±	0,1	**a**	3,1	±	0,2	**a**	1,9	±	0,0	**c**
pdB1G1	1,2	±	0,1	**a**	1,2	±	0,1	**a**	0,9	±	0,0	**b**	0,8	±	0,1	**b**	0,8	±	0,0	**b**
DDG	0,0	±	0,0	**ns**	0,0	±	0,0	**ns**	0,0	±	0,0	**ns**	0,0	±	0,0	**ns**	0,0	±	0,0	**ns**
pdB1G2	3,0	±	0,2	**b**	3,6	±	0,1	**b**	4,2	±	0,2	**a**	4,7	±	0,1	**a**	4,2	±	0,1	**a**
Total Dimers	*50,2*	±	*3,9*	***a***	*41,1*	±	*4,7*	***bc***	*38,0*	±	*4,0*	***c***	*44,7*	±	*3,8*	***ab***	*35,6*	±	*5,8*	***c***
ptC	7,4	±	0,9	**a**	6,6	±	0,5	**a**	5,7	±	0,1	**b**	5,5	±	0,5	**b**	5,0	±	0,2	**b**
ptT2	9,8	±	0,8	**a**	6,9	±	0,4	**b**	4,8	±	0,5	**c**	5,5	±	0,3	**c**	4,2	±	0,4	**d**
ptECG	6,4	±	0,2	**a**	4,1	±	0,2	**b**	4,5	±	0,2	**b**	6,6	±	0,2	**a**	4,4	±	0,3	**b**
Total Trimers	*23,6*	±	*1,9*	***a***	*17,6*	±	*2,2*	***b***	*15,0*	±	*3,5*	***bc***	*17,6*	±	*2,1*	***b***	*13,6*	±	*2,9*	***c***
M+D+T	109,9	±	12,3	**a**	84,4	±	7,9	**a**	74,9	±	6,8	**b**	86,2	±	7,7	**a**	70,3	±	5,0	**b**

In warm vintage, 2009, Site 1 concentrates the greatest amount of procyanidins (109.0 mg/L) compared to Site2, Site 3 , 4 and 5 which all show lower values than 86.2 mg/L. In 2010, the opposite effect can be observed, the lower concentration is found at Site 1 (75.8 mg/L) and Site 5 (72.2 mg/L). Temperate vintage, 2010, shows an increase in total trimeric procyanidins compared to 2009. Monomeric forms are more abundant in 2009 than in 2010, particularly in Site 1 and 2 and in Site 5. In warm mesoclimates the ranges oscillate between 84.4 and 109.0 mg/L and in temperature values range 75.8 and 103.0 mg/L. In late mesoclimate, ranges in 2019 oscillate between 70.3 and 86.2 and in 2010, between 72.7 and 116.0 mg/L. This suggests the amount of procyanidins is not influenced by mesoclimate.

***Table 14**. Procyanidin composition of the wines issued from Site 1 and 2 (Molar) and Site 3, 4 and 5 (Porrera). Vintage 2010. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Location	Site 1		Site 2		Site 3		Site 4		Site 5	
Sample	**wine (mg/L) Vintage 2010**									
Gallic acid	10,2 ± 1,9	**b**	12,7 ± 1,0	**ab**	13,0 ± 0,1	**a**	11,3 ± 0,5	**bc**	9,6 ± 0,5	**c**
Cat	4,9 ± 1,5	**a**	6,8 ± 0,8	**ab**	5,7 ± 0,4	**a**	5,4 ± 1,2	**a**	3,4 ± 0,4	**b**
EC	3,5 ± 1,1	**b**	6,3 ± 0,5	**a**	5,5 ± 0,3	**a**	4,4 ± 0,7	**b**	3,9 ± 0,4	**b**
ECG	0,1 ± 0,0	**ns**	0,2 ± 0,1	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**	0,1 ± 0,0	**ns**
Total Monomers	*18,6 ± 4,4*	***b***	*26,0 ± 2,3*	***a***	*24,3 ± 0,9*	***a***	*21,3 ± 2,3*	***b***	*17,0 ± 1,3*	**c**
pdB1	5,0 ± 1,2	**b**	6,0 ± 0,5	**a**	7,1 ± 0,7	**a**	6,5 ± 1,2	**ab**	4,5 ± 0,3	**b**
pdB2	1,4 ± 0,6	**c**	3,2 ± 0,3	**b**	6,8 ± 0,8	**a**	4,9 ± 1,5	**ab**	3,1 ± 0,3	**b**
pdB3	5,0 ± 1,2	**b**	6,2 ± 0,7	**ab**	7,1 ± 0,7	**a**	6,6 ± 1,2	**ab**	4,5 ± 0,3	**b**
pdB4	9,1 ± 2,1	**bc**	12,3 ± 1,1	**ab**	13,6 ± 0,8	**a**	10,1 ± 1,6	**c**	8,8 ± 0,8	**c**
pdB2MG1	1,4 ± 0,6	**c**	3,2 ± 0,3	**b**	6,8 ± 0,8	**a**	4,9 ± 1,5	**a**	3,1 ± 0,3	**b**
pdB1G1	2,0 ± 0,5	**bc**	2,6 ± 0,3	**bc**	2,8 ± 0,3	**b**	6,6 ± 0,6	**a**	1,8 ± 0,4	**c**
DDG	0,0 ± 0,1	**ns**	0,0 ± 0,0	**ns**	0,2 ± 0,2	**ns**	0,0 ± 0,0	**ns**	0,0 ± 0,1	**ns**
pdB1G2	9,6 ± 0,2	**b**	11,6 ± 0,5	**a**	9,6 ± 0,1	**b**	9,9 ± 0,8	**b**	9,3 ± 0,5	**b**
Total Dimers	*33,4 ± 6,5*	***b***	*45,1 ± 3,6*	***a***	*54,0 ± 4,3*	***a***	*49,6 ± 8,4*	***a***	*35,1 ± 2,8*	***b***
ptC	8,5 ± 0,6	**c**	11,1 ± 0,5	**b**	15,2 ± 0,7	**a**	12,3 ± 1,4	**b**	7,9 ± 0,2	**c**
ptT2	9,6 ± 2,1	**b**	11,7 ± 0,3	**a**	12,3 ± 1,5	**a**	9,4 ± 1,0	**b**	6,7 ± 0,6	**c**
ptECG	5,6 ± 1,7	**c**	9,1 ± 0,9	**a**	10,3 ± 0,7	**a**	6,9 ± 0,8	**b**	5,4 ± 1,2	**c**
Total Trimers	*23,8 ± 4,4*	**c**	*31,9 ± 1,7*	***a***	*37,8 ± 2,9*	***a***	*28,6 ± 3,2*	***a***	*20,1 ± 2,0*	**c**
M+D+T	75,8 ± 15,4	**b**	103,0 ± 7,6	**a**	116,0 ± 8,1	**a**	99,4 ± 13,9	**a**	72,2 ± 6,1	**b**

From this results we can suggest that procyanidin concentration is mostly affected by vintage. This results would also suggest that temperate vintages where maturation of the fruit takes place slowly, the procyanidins can polymerise better than in a warm vintage, where procyanidins might concentrate by dehydration of the berry rather than by a natural evolution of ripeness.

***Table 15**. Summary of the Anthocyanin composition of the wines issued from Site 1 and 2 (Molar) and Site 3, 4 and 5 (Porrera). Vintage 2009 and 2010. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Location	Site 1		Site 2		Site 3		Site 4		Site 5	
2009				**Wine (mg/L) anthocyanins**						
Total G	178,7 ± 6,8	**b**	234,0 ± 3,2	**ab**	238,3 ± 5,6	**a**	214,8 ± 10,3	**c**	241,4 ± 4,2	**a**
Total AG	35,0 ± 1,6	**d**	60,6 ± 1,8	**ab**	58,2 ± 0,6	**a**	62,4 ± 0,5	**b**	68,0 ± 1,5	**c**
Total CG	37,5 ± 2,8	**b**	50,4 ± 6,0	**a**	51,0 ± 1,4	**a**	42,0 ± 8,2	**ab**	46,2 ± 7,7	**ab**
G+AG+CG	251,1 ± 0,5	**c**	345,0 ± 7,1	**ab**	347,6 ± 6,8	**a**	319,1 ± 16,4	**b**	355,5 ± 6,6	**a**
2010										
Total G	138,7 ± 4,2	**b**	151,2 ± 3,0	**a**	136,9 ± 3,6	**b**	141,6 ± 6,3	**b**	141,4 ± 4,9	**b**
Total AG	25,8 ± 0,6	**d**	38,9 ± 1,2	**a**	35,4 ± 0,1	**c**	36,1 ± 0,0	**b**	36,5 ± 1,2	**abc**
Total CG	23,0 ± 0,4	**b**	24,8 ± 0,4	**a**	25,3 ± 0,7	**a**	25,1 ± 1,6	**a**	24,1 ± 0,8	**ab**
G+AG+CG	187,5 ± 5,1	**c**	214,8 ± 4,6	**a**	197,6 ± 4,4	**b**	202,8 ± 7,9	**b**	202,0 ± 6,9	**b**

Table 15 and **Table 16** is a summary of total monomeric, dimeric and trimeric concentrations. In both vintages, catechins and epicatechins are always more abundant species in all sites, with lower epigallocatechin concentration. Site 1 shows the higher monomeric forms amongst all sites. Concerning dimeric forms, procyanidin B4 and B1G2 is the more abundant, being those dimeric forms twice as much as the monomeric forms (in 2010). In 2019 dimeric procyanidin B4 and B2 are more abundant. Trimeric forms range between monomeric and dimeric being ptC, ptT2 and ptECG not significantly different in Porrera in 2010 but all different in 2009.

***Table 16**. Summary of the Procyanidin composition of the wines issued from Site 1 and 2 (Molar) and Site 3, 4 and 5 (Porrera). Vintage 2009 and 2010. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Location	Site 1		Site 2		Site 3		Site 4		Site 5	
2009				**Wine (mg/L) Procyanidins**						
Total Monomers	36,1 ± 2,5	**a**	25,7 ± 1,8	**b**	21,8 ± 3,7	**bc**	23,8 ± 4,6	**b**	21,1 ± 3,3	**b**
Total Dimers	50,2 ± 3,9	**a**	41,1 ± 4,7	**bc**	38,0 ± 4,0	**c**	44,7 ± 3,8	**ab**	35,6 ± 5,8	**c**
Total Trimers	23,6 ± 1,9	**a**	17,6 ± 2,2	**b**	15,0 ± 3,5	**bc**	17,6 ± 2,1	**b**	13,6 ± 2,9	**c**
M+D+T	109,9 ± 12,3	**a**	84,4 ± 7,9	**a**	74,9 ± 6,8	**b**	86,2 ± 7,7	**a**	70,3 ± 5,0	**b**
2010										
Total Monomers	18,6 ± 4,4	**b**	26,0 ± 2,3	**a**	24,3 ± 0,9	**a**	21,3 ± 2,3	**b**	17,0 ± 1,3	**c**
Total Dimers	33,4 ± 6,5	**b**	45,1 ± 3,6	**a**	54,0 ± 4,3	**a**	49,6 ± 8,4	**a**	35,1 ± 2,8	**b**
Total Trimers	23,8 ± 4,4	**c**	31,9 ± 1,7	**a**	37,8 ± 2,9	**a**	28,6 ± 3,2	**a**	20,1 ± 2,0	**c**
M+D+T	75,8 ± 15,4	**b**	103,0 ± 7,6	**a**	116,0 ± 8,1	**a**	99,4 ± 13,9	**a**	72,2 ± 6,1	**b**

4.3 Mesoclimate characterisation for Grenache (El Molar- and El Lloar)

4.3.1 Climatology and soil

The assessment of Grenache vineyards was done during 2010 and 2011. By looking at the climatic diagram for both vintages, temperatures were higher in 2011 during the ripening period (September), while in 2010 the temperatures were more moderate, averaging up to 5ºC less than in 2011 (**Figure 46**). Maximum temperatures in 2010, in the LO plot, reached values slightly below that of the EM plot, with a peak in July. In contrast, in 2011 the highest temperatures appeared one month later than their peak in 2010, reaching markedly high values of vapor pressure deficit (VPD) at the end of August and September, corresponding with the grape ripening period. The maximum temperature in 2011 remained high for several months with no variance between plots. The annual rainfall in 2010 was lower by 75mm compared to 2011, with low rainfall between June and October, being almost null values in the months of August and September. Vintage 2010 did not carry continued VPD values as high as in 2011 in the same period. Thus, 2010 was defined as milder vintage. As expected, the crucial months defining the characteristics of the vintage are July, August, September and October – the period between veraison and ripening. The 2010 vintage was characterized by a heterogenic distribution of rainfall and a lower vapor deficit pressure than 2011.

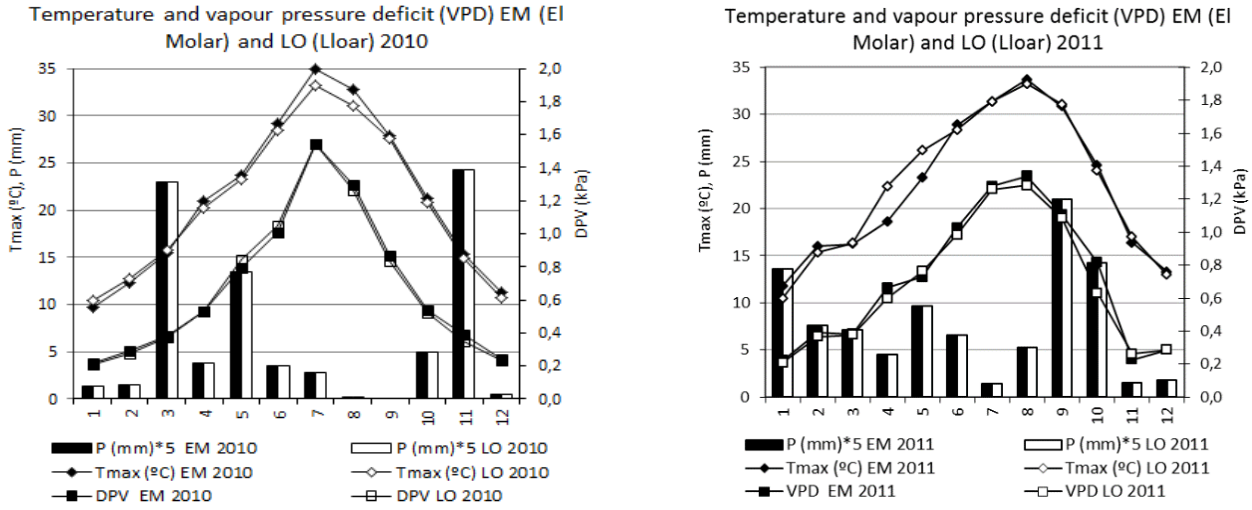

***Figure 46**. 2010 and 2011 climatology in EM (El Molar) and LO (El Lloar). Tmax (maximum Temperature), P (rainfall) and DPV (vapor pressure deficit).*

Concerning the soil structure, the plot of EM features a similar texture between the soil and subsoil layers. EM gravely elements in both soil and subsoil ranged between 35-40%, while the remaining percentage corresponds to fine particles giving a clay loam texture in USDA classification terminology (**Table 17**). In contrast, the LO subsoil contains less clay and is much richer in silt. The soil texture in the first layer, in LO, is clearly gravely, whereas in its

subsoil silt is predominant. In LO, the soil is much more stony, with a clay content (25.3%) higher than that of the EM plot (5.3%). This heterogeneity results in the two plots having different water drainage characteristics, explaining the decrease in leaf area of LO vines at the end of the growing season (**Table 17**). The VPD in 2010 was lower at ripening (September and October), reflecting lower temperatures. Toghether with a high VPD, this decrease in leaf area lasted until two weeks before harvest, coinciding with the period of grape maturation.

***Table 17**. Soil and Subsoil Texture.*

		< 2mm	>2mm	% sand	% silt	% clay	USDA-Classification
EM	Soil	59.4%	40.6%	46.3	48.3	5.3	Sandy loam
	Subsoil	65.8%	34.2%	40.0	54.7	5.3	Sandy loam
LO	Soil	36.4%	63.6%	42.0	32.7	25.3	Silty loam
	Subsoil	70.3%	29.7%	25.3	70.0	4.7	Silty loam

4.3.2 Phenology and vegetative growth

In general, the evolution of TLA (total leaf area) was similar in both vineyards (EM, El Molar and LO, El Lloar) in the temperate year (2010), showing the same trend, with differences only at pea size (**Figure 47**). The leaf area of the two plots evolves differently in 2011; in the LO plot it is observed more growth than in the EM plot. In 2011 the greater leaf area achieved in LO during veraison, induced by the continuous rainfall during the spring combined with extreme temperatures during maturation, resulted in a greater decrease in leaf area compared to the previous year. At ripening no leaf area differences were observed, in either vineyard, regardless of the vintage. In 2010 the LO plot grew a slightly larger leaf area than the EM vines, given the scarcity in the distribution of rainfall during the spring. In 2011, from veraison to ripeness, the attached graph slopes of leaf size show a steep decrease compared to 2010.

***Table 18**. Phenological stages and dates for Grenache in two vintages, 2010 and 2011.*

2010	Fruit set	Pea size	Veraison	Harvest	Leaf drop
EM	1-Jun	21-Jun	22-Jul	06-Sep	04-Nov
LO	10-Jun	05-Jun	27-Jul	12-Sep	04-Nov
2011					
EM	4-Jun	19-Jun	18-Aug	04-Sep	01-Oct
LO	11-Jun	14-Jun	21-Aug	29-Aug	29-Oct

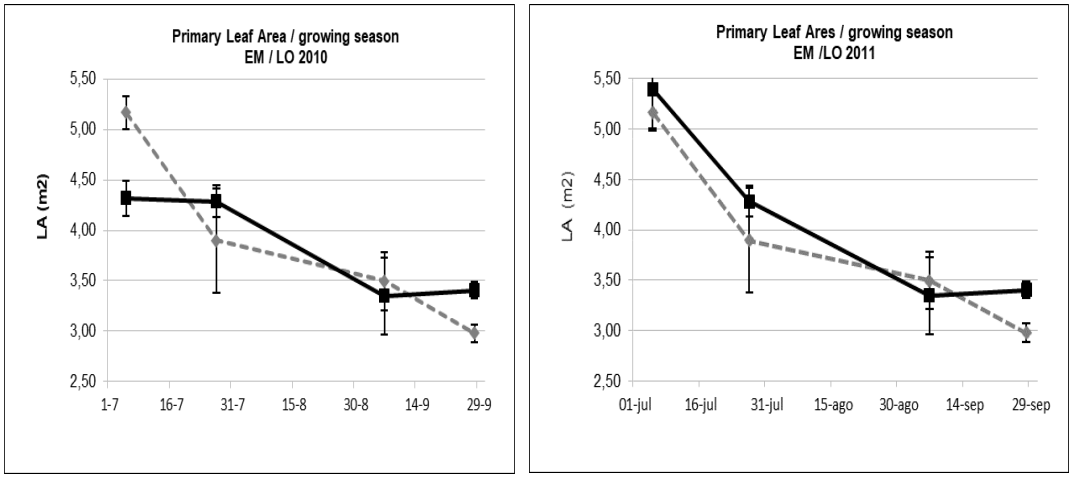

***Figure 47**. Primary Leaf Area during the growing season in El Molar (EM) and El Lloar (LO) for Grenache. EM (black) and LO (grey)*

During 2010 and 2011, leaf area (LA) at the phenological stages of pea size (PS), veraison (V), final ripening (RP) and post-harvest (PH) was measured. Total leaf area (TLA) within parcels did not differ significantly in the temperate year. In the drier vintage, however, vines from LO developed more leaf area than those growing in the south-facing terraces at EM. Nevertheless, the total leaf area before harvest was similar. The heterogeneity in the soil profile at the LO location could likely induce a variation in the drainage capacity, affecting the vine growth (TLA).

***Figure 48**. Grenache Vineyard in el Molar (Site 6, EMGRE)*

***Figure 49**. Grenache Vineyard in El Lloar (Site 7, LLOGRE).*

4.3.3 Leaf water potential

In 2010, the difference between EM and LO is most marked at PS (LWP measured at 5:30) and at PH (LWP measured at 11:30). In contrast, around maturity (RP), the most notable differences are shown to the extent of Ψ 11:30. In all phenological stages, the tendency in the last measure, is to recover the potential, reaching less negative values, thus indicating less plant stress. During ripening (RP) and at 11:00, point of the day of greatest water stress, LO gives more negative values than EM, although the differences between plots are very small.

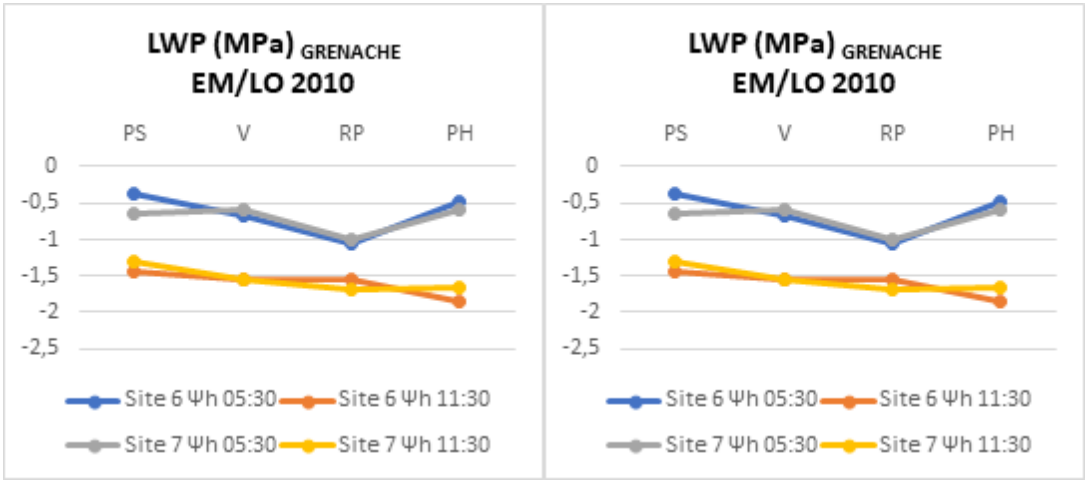

***Figure 50**. Leaf water potential in El Molar (EM) and El Lloar (LO) for Grenache in 2010 (left) and 2011 (right).*

The water potential of 2011 was not so different between plots if we compare with 2010. It should be noted the great difference between the different phenological stages where particularly harvest date was delayed in 2011. During pea size (PS), the water potential is less negative, but during veraison (V) and maturation (RP), coinciding with the month of highest VPD, the plant is more affected by water stress (more negative values). In 2010,

stress was more pronounced during RP and the vines did not achieve recovery shown by a decrease in LWP. In both vintages, Grenache never showed lower LWP than Carignan.

4.3.4 Leaf temperature

The leaf temperature with respect to the external temperature shows a very similar evolution at 8:00 am along with the vegetative cycle. At noon the differences between temperatures are different in PS and V but not in RP and PH. This would indicate that the refrigeration of the plant given through the stomatal opening is minimal from mid-summer until the end of the ripening period. The measurements have only been carried out in 2011 (warm vintage). Even so, comparing with the leaf temperature measurements carried out in Carignan, there are less differences between T_{leaf} and T_{air} in Garnacha.

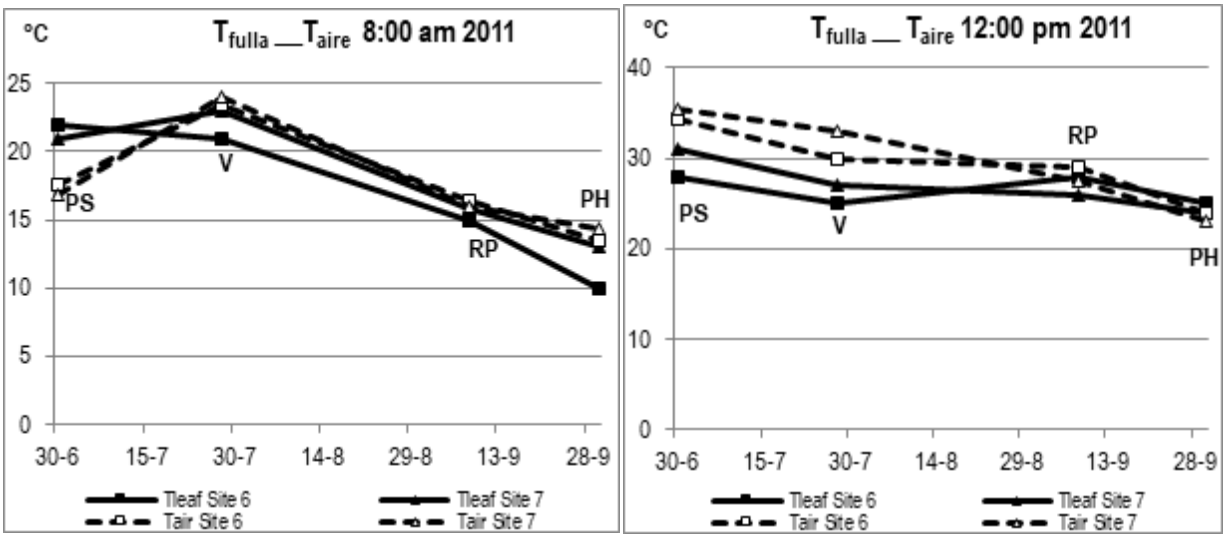

***Figure 51**. Measuremenets of leaf temperature (T leaf) and air temperature (T air) in Porrera during the vegetative cycle 2010.*

4.3.5 Stomatal conductance

The evolution of stomatal conductance (g_s) in cultivar Garnacha shows a very different behaviour depending on whether the year is warmer or more temperate (**Figure 52**). In a warmer year (2011) a more constant daily evolution is observed, showing less stomatal conductance as the hours of the day progress, the highest values being at the beginning of the day with values ranging between 100 and 200 mmol m^{-2} s^{-1}. On the other hand, in temperate year (2010) the conductance in Grenache oscillates depending on the time of day, always showing a lower conductance at noon and a recovery at the end of the day. Values at noon are below 100 mmol m^{-2} s^{-1} on V and RP. This oscillation would indicate a stomatal closure and opening as a function of external conditions, thus showing a more adaptive physiological response to environmental conditions.

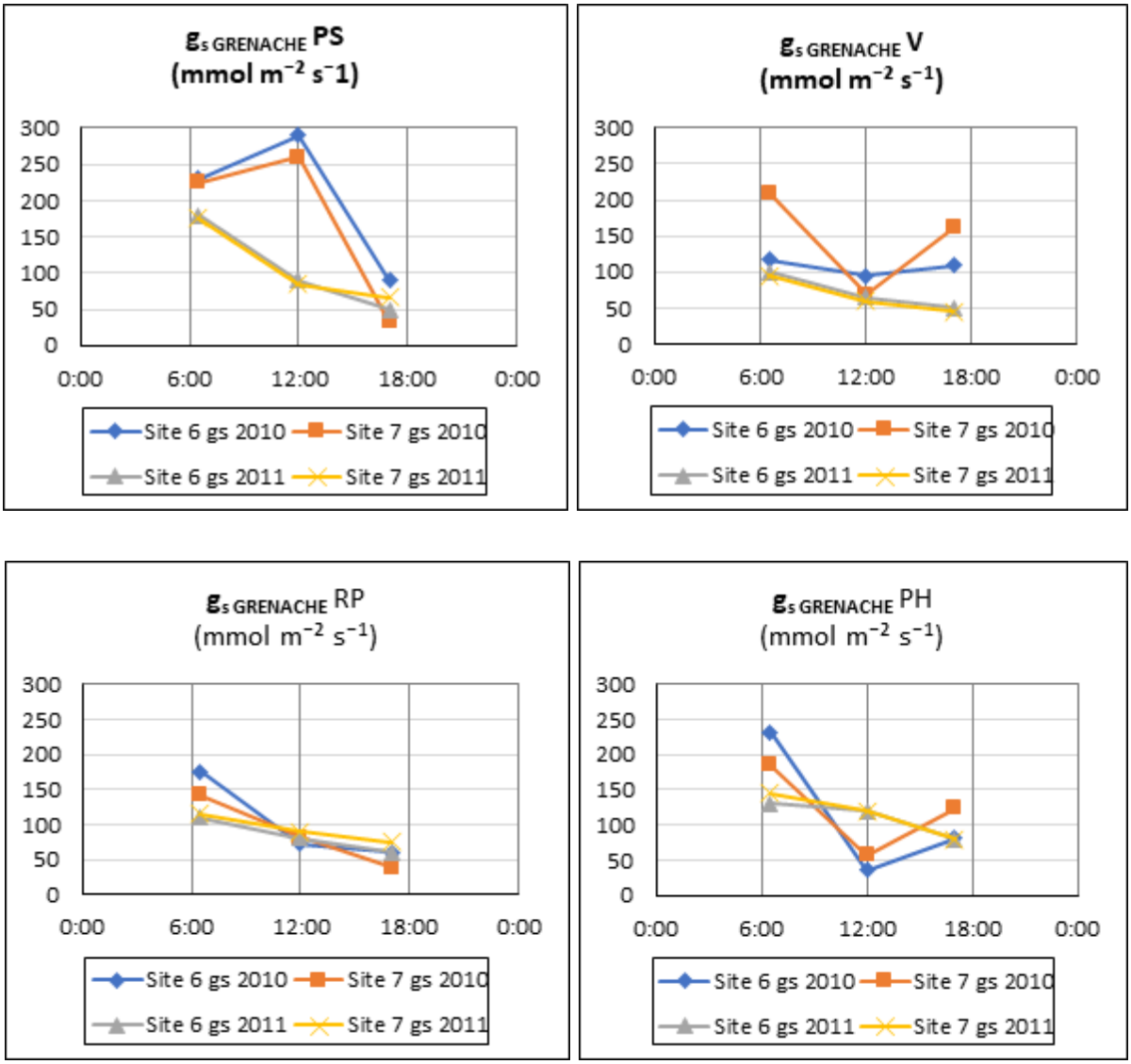

***Figure 52**. Stomatal conductance (g_s) measured in cv. Grenache in 2010 and 2011 vintages. Site 6 (Grenache in El Molar, EMGRE) and Site 7 (Grenache in El Lloar, LLOGRE).*

4.3.6 Acid Abscisic composition

ABA was determined through the growing cycle in 2011. Acid abscisic (ABA) concentration in Grenache at PS shows lower values than any other phenological stage with no significant differences at pea size. At veraison (V) ABA concentration increases considerably at Predawn and Midday. At RP, acid abscisic maintains the high concentrations acquired during veraison (V). ABA only decreases after harvest. Values obtained at PH might show high variability within the plant, as most of the leafs have lost turgency and yellowing was quite visible.

***Table 19**. Values for abscisic acid concentration (ng/g) (ABA) for sites 6 and 7 at 4 different stages of growth -pea size (PS) and veraison (V). ripeness (RP) and post-harvest (PH) at Predawn and Midday. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

	Pea Size (PS)		**Veraison (V)**	
Site	**[ABA] Predawn**	**[ABA] Midday**	**[ABA] Predawn**	**[ABA] Midday**
6 (Molar)	97.8 (3.3)	185.7 (17.5)	238.3 (5.3)	466.4 (22.5)[b]
7 (Lloar)	73.6 (7.5)	153.1 (9.8)	240.7 (9.0)	546.3 (15.2)[a]
	Ripeness (RP)		**Post-Harvest (PH)**	
Site	**[ABA] Predawn**	**[ABA] Midday**	**[ABA] Predawn**	**[ABA] Midday**
6 (Molar)	286.9 (22.2)[a]	462.2 (13.8)	204.8 (11.5)[b]	428.8 (26.6)[a]
7 (Lloar)	148.3 (5.5)[b]	477.0 (48.1)	237.7 (11.4)[a]	234.1 (18.1)[b]

4.3.7 Grape and wine composition

Grape composition: our research indicates significant differences between the two plots in both years of study. For the EM plot, both vintages resulted in higher Brix values. The LO plot in 2011 had a particularly higher value of TTA compared to the EM plot, but no differences in pH. Concerning phenolic composition, both years the EM plot showed the highest content of ANT T (Total anthocyanins), ANT E (Extractable anthocyanins), TPI (Total polyphenol index) and DMAC. It should be emphasized in the warmest year (2011) that differences between plots equalized, but were exacerbated in 2010. The EM plot's berry size was similar in both years, but the LO plot berry size differed each year depending on climate, and thus the final composition of the wine differed from one year to the next.

Table 20. *Grape must composition and berry weight. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

		Berry weight (g)	Brix	TTA (g/L)	pH
EM	2010	1.44 (0.05) b	27.4 (0.0) a	4.6 (0.1) a	3.55 (0.01) a
LO	2010	1.74 (0.01) a	26.9 (0.1) b	4.2 (0.1) b	3.45 (0.02) b
EM	2011	1.40 (0.02) a	27.5 (0.5) a	4.3 (0.2) b	3.40 (0.06) a
LO	2011	1.28 (0.07) b	26.3 (0.4) b	5.6 (0.1) a	3.50 (0.05) a

Table 21. *Grape phenolic composition. . [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

		ANT T (ppm)			ANT E (ppm)			TPI			DMAC (ppm)		
EM	2010	661.5	(39.4)	a	452.1	(8,1)	a	65.7	(3.2)	a	103.2	(5.2)	a
LO	2010	520.3	(41.8)	b	359.3	(23,5)	b	54.2	(4.3)	b	82.6	(4.8)	b
EM	2011	557.7	(103.5)	a	455.6	(57,0)	a	69.0	(3.9)	a	235.9	(20.3)	a
LO	2011	479.5	(43.9)	a	392.0	(43,9)	a	64.0	(1.6)	a	224.0	(28.5)	a

Rainfall occurring during spring affects the vegetative growth, over two different climatic years. Temperatures during the ripening period, proved crucial, particularly the vapor pressure deficit. In the case of Grenache, grape composition is clearly affected by weather conditions in early September in the area studied, with major differences in phenolic composition between plots during the cooler year. The warmer year did not change the quality of grape composition, must, or polyphenol composition as much as the temperate. A similar trend is observed in the wines, in which composition is similar between plots, suggesting that both, the climatology of the year and the soil profile have a higher impact on the quality of grapes than the topographical situation. The content of flavan-3-ol and tannins in the wines depends on the type of plot only in temperate years, while in warm years synthesis occurs equally regardless of the vineyard parcel.

***Table 22**. Wine composition, ABV: alcohol by volume, TTA: total titratable acidity, and pH. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

		% ABV		TTA (g/L)		pH	
EM	2010	16.1 (0.1)	a	5.5 (0.0)	a	3.55 (0.03)	a
LO	2010	15.5 (0.4)	a	5.0 (0.4)	b	3.64 (0.08)	a
EM	2011	15.5 (0.1)	a	5.3 (0.4)	a	3.65 (0.16)	a
LO	2011	15.1 (0.2)	a	5.3 (0.2)	a	3.50 (0.07)	a

***Table 23**. Wine composition, ANT T: total anthocyanins, DMAC, TPI (Total polyphenol index) and tannins. . [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

		ANT T (ppm)				DMACA (ppm)				TPI				Tannins (g/L)			
EM	2010	239.9	±	22.5	a	324.8	±	47.0	a	47.0	±	3.0	a	1.91	±	0.05	a
LO	2010	186.8	±	23.8	b	274.2	±	64.8	a	38.2	±	3.4	b	1.33	±	0.12	b
EM	2011	361.4	±	72.1	a	376.3	±	94.4	a	40.3	±	6.0	a	1.56	±	0.33	a
LO	2011	355.7	±	47.4	a	412.2	±	36.3	a	45.9	±	4.1	a	2.00	±	0.61	a

Wine composition: for both vintages the highest concentration of anthocyanin was found in the EM treatment, showing major differences from the LO plot in 2010. The smaller the berry size, the higher the ANT T and DMACA, regardless of vintage. The greatest differences in concentration occurred during the temperate year (2010). The greatest amount of tannin concentration resulted from smaller berries. The total polyphenol index does not differ significantly between plots and years. Lower polymerization of the flavan-3-ol units were a function of the smaller berry size.

4.4 Vineyard classification based on water stress assessment on Carignan grapevines by using classification and regression trees

This paper has been published as a result of this thesis:

Received: 23 August 2020 | Revised: 7 March 2021 | Accepted: 10 March 2021

DOI: 10.1002/pld3.319

ORIGINAL RESEARCH

American Society of Plant Biologists SEB WILEY

Water stress assessment on grapevines by using classification and regression trees

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Funding information
This research was funded by the University Rovira i Virgili, Biochemistry Department, Viticulture Research Group. Partial support for this work has been received from the Agency for Management of University and Research Grants (AGAUR) of the Government of Catalonia (grant 2017 SGR 705).

Abstract

Multiple factors, such as the vineyard environment and winemaking practices, are known to affect the development of vines as well as the final composition of grapes. Water stress promotes the synthesis of phenols and is associated with grape quality as long as it does not inhibit production. To identify the key parameters for managing water stress and grape quality, multivariate statistical analysis is essential. Classification and regression trees are methods for constructing prediction models from data, especially when data are complex and when constructing a single global model is difficult and models are challenging to interpret. The models were obtained by recursively partitioning the data space and fitting a simple prediction model within each partition. The partitioning can be represented graphically as a decision tree. This approach permitted the most decisive variables for predicting the most vulnerable vineyards and wine quality parameters associated with water stress. In Priorat AOC, Carignan grapevines had the highest water potential and abscisic acid concentration in the early growth plant stages and permitted vineyards to be classified by mesoclimate. This information is useful for identifying which measurements could most easily differentiate between early and late-ripening vineyards. LWP and T_s during an early physiological stage (pea size) permitted warm and cold areas to be differentiated.

KEYWORDS

ABA, anisohydric, carignan, classification and regression trees, isohydric, water stress

4.4.1 Introduction

Water stress on vine plants induces the synthesis of secondary metabolism. Around veraison, water deficit stress causes a significant increase in the abscisic acid (ABA) level in fruit zone leaves (Okamoto et al., 2004) and berries (Coombe and Hale, 1973; Düring and Allenweldt, 1980). ABA plays an important role in the regulation of growth and the ripening of vines. Lack of water in the soil and elevated temperatures induce the synthesis of ABA in the roots, followed by its translocation to the leaves, where it rapidly alters the osmotic potential of stomatal guard cells, causing them to shrink and the stomata to close. Stomatal closure reduces transpiration and thus prevents further water loss from the leaves during periods of low water availability. Around veraison, ABA levels in grapes increase significantly, along with the stimulation of ripening and phenolic synthesis, but decrease during the final stage of berry ripening (Palejwala et al., 1985; Soar et al., 2006; Wheeler et al., 2009; Bondada and Shutthanandan, 2012). Abscisic acid may be translocated from the sites of biosynthesis, such as roots and leaf vascular tissues, to the guard cells. Recent identification of multiple transmembrane ABA transporters indicates that the movement of this hormone within plants is actively regulated in an intercellular network (Kuromori et al, 2018).

Regulation of water deficits has often been used to balance grapevine vegetative and reproductive growth to control berry quality (Chaves et al., 2010). Analysis of the phenolic composition in wine is essential for establishing quality parameters related to water stress, as some studies have shown that ABA is involved in the mechanisms controlling the synthesis of anthocyanins and promotes the synthesis of tannins accumulating in skin (Lacampagne et al., 2010) ABA synthesis depends on different factors promoting water stress; plant water physiology is affected by various environmental factors (e.g., topography, soil water-holding capacity, temperature, rainfall, and vapor deficit pressure), plant vigour, and cultural practices, such as irrigation techniques and fertilization programs (Jackson and Lombard, 1993; Downey et al., 2004) and by scion/rootstock interaction with soil type (Lavoie-Lamoureux et al., 2017), Grenache is highly influenced by vigour, because anthocyanin accumulation is favoured in balanced, high-vigour vines, whereas in Carignane, the anthocyanin content varies under the combined effects of vigour, rootstock, berry size, and vintage (Edo et al., 2014) .

Appropriate statistical tools are required for identifying the factors that have the strongest effects on quality and stress during growth (plant) and maturation (grape). Predictors, such as linear or polynomial regressions, are global models, where a single predictive formula is applied over the entire dataset. However, when the data interact in complex, nonlinear ways, assembling a single global model is challenging. Classification-type problems can be resolved when a categorical dependent variable (e.g., class and group membership) is predicted from one or more continuous and/or categorical predictor variables. Generally,

the purpose of analyses involving tree-building algorithms is to determine a set of *if-then* logical (split) conditions that permit accurate prediction or classification of the data.

The aim of this study was to evaluate the efficacy of a multivariate nonparametric technique of classification and regression trees (CART) for identifying and selecting the most important factors affecting water stress in vineyards with a heterogenic orography (e.g., leaf water potential (LWP), concentration of ABA, surface leaf temperature (T_s)); analyse the effect of these interactions on final grape and wine quality (e.g., composition of anthocyanins and procyanidins); and improve the rapidity with which ABA can be measured in grapevine leaves. The heterogeneity of the vineyards in the Priorat wine region requires the collection of a considerable amount of data and more robust statistical tools to better understand the factors affecting water stress in vineyards. Because of the increasing drought and higher temperatures occurring in the Priorat, the Priorat is highly vulnerable to future climate change. Here, we explore applications of multivariate nonparametric classification techniques such as CART, a type of decision tree technique (Breiman et al., 1984), given that traditional methods are not appropriate for analyses because of the characteristics of the variables studied.

4.4.2 Results

LWP and ABA measurements are shown in **Table 24** and **Table 25**. After characterizing differences in variability through a non-parametric Kruskal-Wallis test (**Table 26**) at a significance level of 5%, Pearson correlations between the measured variables and their significance (**Table 27**) were calculated. The classification of sites was captured by the Classification and Regression Trees (CART) to help identifying key variables in the data. The most meaningful predictors were used to create the tree . Plant, grape and wine data were collected to evaluate the interactions. However, to obtain reliable classification and regression trees, a previous selection of nodes and child's was completed using the easiest-to-measure variables in the field and the easiest-to-analyse variables in the laboratory. Each round of data is known as 'nodes'. Each node will have an *if-else* clause based on a labelled variable. Based on that question each instance of input will be directed/routed to a specific leaf-node which will tell the final prediction. The tree depth is chosen as the most number of levels desired in the decision tree. The first node is split based on the most important predictor, then the following child nodes are broken down to separate out the next variable. Entering a value the program sets the minimum number of cases an internal node to be split. 3 times terminal node limits allow a reasonable number of splitters.

CART: water stress and plant growth

Plant growth parameters that differed significantly (p-value ≤0.05) between plots were berry weight and total leaf area/kg (TLA/kg) at the veraison (V) and ripening (RP) stages. Water stress indicators that differed significantly between plots were LWP and [ABA] at pea size (PS) and veraison(V) and surface temperature (T_s) at pea size (PS). Pearson correlations revealed that LWP at PS measured at 8:00, ABA at V measured at 14:00, and T_s at PS measured at 8:00 were negatively correlated with the synthesis of anthocyanins in wine for all anthocyanin families (acylated and non-acylated). LWP and T_s showed stronger correlations when these parameters were measured earlier in the day (8:00) or at the beginning of the vegetative cycle (PS). The same variables—LWP at PS measured at 8:00, ABA at V measured at 14:00, and T_s at PS measured at 8:00—were positively correlated with TLA/kg V.

As a result from this the CART, LWP at PS measured at 8:00 was the most important predictor allowing to create the first node that separated early mesoclimates (nodes 6 and 7) from late mesoclimates (nodes 4 and 5). Nodes 2 and 3 were dependent on ABA at PS (late mesoclimate) and V (early mesoclimate). However, obtaining a partition of the five sites [ABA] at V was decisive and resulted in the generation of nodes 8 and 9. As a consequence, the sites with the highest probability of being classified with LWP values ≤ -0.863 (8:00 at PS) were the parcels located in the town of Molar (sites 1 and 2). Hence, site 1 had levels ≥ ABA 175.9 ng/g (14:00 at V) (**Figure 53**). Site 3, within the late mesoclimate area, had a lower probability of having ABA ≤ 183.9 ng/g (morning at pea size) PS. Fewer factors differentiated site 3 (grey) from the other sites; it was thus separated in an early node as in sites 1 and 2 (blue and red) of the early mesoclimate area (**Figure 53**)

CART: ABA, LWT, and T_s

The most significant variables for characterizing and classifying the observations were [ABA], LWP, and T_s. T_s was selected given that it had a direct relationship (positive Pearson correlation) with the vegetative growth parameters of TLA/kg and berry size. The Pearson correlation produced a clear classification tree (**Figure 54**) based on the T_s, at the root node, it generated three child nodes (2, 3, and 4). This first classification by T_s at PS measured at 7:00 resulted in a purity of 100% for site 4, but the T_s at PS measured at 12:00 was clearly the most important variable for sites 5 and 6 under a second child node classification. However, the early sites (1 and 2) were differentiated by [ABA] at PS measured at 8:00.

Although many authors have described the effect of T_s on the quality of grapes during the ripeness period (Spayd et al., 2002; Van Leeuwen et al., 2009; Greer and Weedon, 2013), the analysis of the tree shows the magnitude of the effect of T_s from the early stage of PS. Measurements taken at 8:00 at PS were more likely to have values of T_s ≤ 22.0ºC in the

late mesoclimate area. Child node 4 indicates that sites 1 and 2 had a high probability of being classified within the temperature range 22ºC ≤ T_s ≤ 24ºC (8:00 at PS). Using the CART greatly facilitates the characterization of the importance of the classification of vineyards, especially in the late area (sites 3, 4, and 5). Furthermore, sites 3 and 5 were located in equivalent positions in the tree (purity 50%); thus, the differentiation of both plots from other plots depended on the same factors. Remarkably, both site 3 and site 5 had similar TLA and thus greater water loss. (**Figure 54**)

CART: anthocyanins in wine quality

In this CART analysis, Pearson correlations of plant parameters and wine composition in each site were calculated. Both LWP at PS measured at 8:00 and LWP at V measured at 14:30 were correlated with ANT (mg/L), A-G (mg/L), and A-AG (mg/L). However, lower correlation coefficient values were obtained for LWP at V measured at 14:30pm. Despite the difficulty of establishing direct links between plant parameters (TLA/kg at V) and wine composition (anthocyanins), robust correlations were found for T_s at PS measured at 7:00 and wine anthocyanins (non-acylated and acylated). The most significant relationship was for the correlation between TLA/kg V and A-AG (mg/L).

Based on the easy-to-measure parameters in the vineyard, such as T_s and the ratio of leaf area and production at V (TLA/kg V), we could characterize the relationship between the water status of plants and plant growth to the quality of the final wine product. This classification of plots allowed us to determine patterns of heterogeneity between plots. Thus, the CART classifies sites through the nodes to distinguish among different vineyards. (**Figure 55**)
The tree shows that LWP (node 1) at PS permitted the differentiation of early (EM) and late (PO) sites. Values within the range -1.45 ≤ LWP ≤ -0.862 described the late ripeness sites (4, 5, and 6), while the range -0.863 ≤ LWP ≤ -0.290 classified the warmest sites (1 and 2). In the late mesoclimate area (node 2), sites were separated by anthocyanins; sites 3, 4, and 5 were classified together by node 5 and were primarily influenced by the LWP at 14:30 in V. This finding suggests that the topography of the vineyard location, as well as the climate and soil type, had an important influence on wine quality. However, the parameter that classifies vineyards was ABA at 14:00 V (≤ 175.9 ng/g) by node 3 and was necessary for divided sites 1 and 2 (early mesoclimate).Thus, LWP did not affect the phenolic content of the wines.

4.4.3 Discussion

Measurements of the distribution of soil water revealed that the differences detected among the five sites reflected heterogeneity in soil particle size, depth, and texture. Sites 1 and 2 (El Molar) on a clayey soil had a higher water-holding capacity, than that of sites

3, 4 and 5 (Porrera), which were steep with more stones and soil was primarily composed of larger elements. Thus, the vines in the town of El Molar (site 1 and 2, early mesoclimate) had more available water than those in Porrera (sites 3, 4 and 5, late mesoclimate), despite the lower rainfall recorded during the cycle. Pre-dawn leaf water potential (PLWP) reflects soil water availability as perceived by the plant and midday leaf water potential (MLWP) measures leaf water potential under maximum daily water demand. Therefore the higher soil water content at sites 1 and site 2 led to more vigorous plants because LWPs were less negative. In Porrera, because of the lower water retention in soils, the plants had more negative LWPs than those in Molar. In addition, at approximately the pea size phenological stage, water transpiration by leaves was higher and LWPs showed more negative values because of the low soil water content in the stony and poor soil. It is known that *Vitis* genotypes show either an isohydric or anisohydric response to water stress. In isohydric cultivars, strong control of stomatal conductance by ABA reduces transpiration, obviates decreases in water potential, and delays the onset of stress tolerance mechanisms. In contrast, weak ABA control of stomatal closure does not avoid midday decreases in water potential in anisohydric grapevines (Lovisolo et al., 2010). In addition, during periods of low water availability and higher transpiration water demand, many authors have observed that a hydraulic signal can also have a controlling effect on stomatal conductance, and this also relates to both patterns, isohydric species maintain relatively stable LWPs precisely because of their more strict stomatal control, whereas anisohydric species would show a looser regulation of transpiration. What is more, the degree of isohydry can be related to a reduced soil water availability (lower, more negative soil water potential, Ψ_{soil}) may affect plant conductance in two ways, by lowering its hydraulic conductance (K_H) and/or its leaf conductance (g_{Leaf}). These reductions, have opposite effects on the water potential difference through the plant ($\Delta\Psi = |\Psi_{Leaf} - \Psi_{soil}|$), whereas lower K_H increases $\Delta\Psi$, lower g_{Leaf} decreases $\Delta\Psi$ (Martínez-Vilalta et al. 2014, Martínez-Vilalta and García-Forner, 2017). Thus, there is a tight coordination between hydraulic and water vapour transport at the plant level (Sperry and Love, 2015).

Parameters that best discriminated between sites were LWP and ABA content, followed by berry size and anthocyanin concentration. Around veraison, higher correlations between LWP and ABA content were obtained. After analysis of the Pearson correlations, the best results were obtained for the veraison phenological stage where vapor pressure deficit (VPD) is lower. ABA concentrations in Carignan vines at different sites (early (1 and 2) and late (3, 4, and 5)) are shown in **Table 25**. Higher concentrations of ABA were observed in all vineyards when measurements were taken at noon. This observation reflects increased water stress in all plots and confirmed measurements of LWP. It also established a direct correlation between the concentration of ABA and LWP (R^2=0.918). The strongest correlations were observed for the first measurements in the morning, while measurements at noon showed greater dispersion, R^2 (0.7175). Thus, the CART analysis

could distinguish among sites of the later mesoclimate region based on ABA at pea size stage.

In **Figure 53**, PLWP at pea size separated sites within mesoclimate and reached values of -0.86 for the early and -1.45 for the late mesoclimate. Around veraison, ABA concentration classified vineyards in the warmest area with values of 258 ng/g in site 1 and 175 ng/g in site 2. In the coldest area, the values were lower and did not separate at such wide intervals. At site 3, ABA concentration did not exceed 183 ng/g at pea size; instead, values were higher in sites 4 and 5 but did not differentiate vineyards. Values for these plots at veraison were lower than in pea size; site 3 had values as high as 164 ng/g, and site 5 had values as high as 188 ng/g. The three most similar sites in ABA at veraison at noon were sites 2, 4, and 5. Thus, the ABA concentration at veraison is important for differentiating most of the plots, including sites 1, 2, 4, and 5.

In **Figure 54**, T_s at pea size measured at predawn permitted separation by temperature ranges and isolated site 2 with temperatures between 19.8 and 22°C. The early sites were separated by ABA at pea size at predawn (with higher values in site 2, considering that site 1 had a rocky soil, while site 2 was composed by finer elements). Plots of the coldest area were only separated by T_s at pea size at noon. Site 5 was located at higher elevation and experienced higher temperatures at noon (36.6°C) than site 3 (35.2°C). T_s at veraison did not provide useful information because the plots experienced similar levels of stress. Thus, the characterization of the plots by T_s can be predicted at pea size but not around veraison.

In **Figure 55**, PLWP at pea size separated sites with different mesoclimates. Sites 1 and 2 differed in ABA around veraison at noon (**Figure 55**). The ABA concentration in site 1 was twice that of site 2; thus, these plots did not differ in the concentration of anthocyanins unlike the colder sites. Sites in the cold mesoclimate were classified by the anthocyanins in wine. Although there was a strong correlation with anthocyanins in grapes, wine correlated with other variables (as evidenced by Pearson correlations greater than 0.7). Plants at site 2 were the least vigorous with anthocyanin values less than 339 m/L. Because plants at sites 3 and 4 showed more vigour, the effect that distinguished the plots was MLWP at veraison, as the water stress was increased in site 3 (LWP of -1.82) and site 4 (LWP of -1.6).

Even if the action of ABA in occlusive cells is complex and not yet fully understood, *Vitis* genotypes apparently exhibit different levels of drought adaptation that differ in key steps involved in ABA metabolism and signalling (Rossdeutsch et al., 2016). In general, *Vitis vinifera* varieties, displayed more pronounced responses to water-deficit in comparison to other *Vitis* genotypes. Moreover, Dal Santo et al. (2016) proposed a cause-effect link between the physiological grapevine plant conditions and the intensity of the gene expression changes. Finally, in regards to grape composition, many key genes

(VvMybA1 and VvUFGT) of the flavonoid biosynthetic pathway are also up-regulated during ripening, resulting in a berry quality increase (Ferrandino and Lovisolo, 2014). ABA accumulation and the induction of flavonoid biosynthesis increase the quality of berries by facilitating the accumulation of secondary metabolites, especially polyphenols. Under water stress, polyphenolic concentrations increase in berries both in isohydric varieties, such as *Grenache* (Coipel et al., 2006), *Tempranillo* (Santesteban et al., 2011), *Manto negro* (Medrano et al., 2003), and in anhysohydric varieties, such as *Cabernet Sauvignon* (Kennedy et al., 2002; Bindon et al., 2008), *Cabernet Franc* (Matthews and Anderson, 1988), and *Muscat of Alexandria* (Dos Santos et al., 2007), with different temporal dynamics related to ABA induction. Aquaporins are another target for ABA to regulate both water and carbon fluxes. ABA affects aquaporin regulation in response to abiotic stresses (Kaldenhoff et al., 2008) by modulating their gene expression and protein abundance or activity, affecting in cellular water relations and cell metabolism in response to water stress. Aquaporins can be modulated at several levels, via transcription, translation, trafficking and gating (opening and closing of the pore) and by environmental and developmental factors (Chaumont and Tyerman 2014), such as: irradiation (Prado et al. 2013, Lopez et al. 2013), transpiration (Sakurai-Ishikawa et al. 2011, Laur and Hacke 2013), circadian rhythms (Hachez et al. 2008), abscisic acid (ABA) feeding (Shatil-Cohen et al. 2011, Pantin et al. 2013), auxin feeding (Péret et al. 2012) and shoot wounding (Sakurai-Ishikawa et al. 2011, Vandeleur et al. 2014). Coupled with that, Castellarin et al., (2007) showed that water stress favoured the accumulation of more hydroxylated and methylated anthocyanins (peonidin 3-O-glucoside and malvidin 3-O-glucoside). In addition, the degradation of anthocyanin would probably be induced by high temperatures with an oxidative stress leading to the formation of H_2O_2, with the subsequent induction of peroxidases and of oxidoreduction enzymes (Mori et al., 2007). In contrast, little is known about the impact of temperature on proanthocyanin accumulation in grape skins; berries are able to compensate the initial effects of temperature on proanthocyanin biosynthesis resulting in similar concentration of proanthocyanin at harvest (Cohen et al., 2012).

Overall, the effect of variables on the classification of the trees was closely tied to the water scarcity of the plants. In viticulture science it is of particular importance to evaluate whether the relationships between physiological parameters fitted to data through these powerful statistical methodologies. In addition, some authors (Brillante et al., 2017) have shown that well-trained machine-learning models can be used to capture the essential relationships between plant physiology and the environment. As an example, Brillante et al. (2016) have for the first time modelled grapevine water stress. This models will be important to design experiments and provide with validation tests to demonstrate the efficiency of the models.

Table 24. *Values of predawn leaf water potential (PLWP, ΨPLWP (MPa)) and midday leaf water potential (MLWP, ΨMLWP (MPa)) for sites 1, 2, 3, 4, and 5 at two different stages of growth—pea size (PS) and veraison (V)—at Predawn and Midday.. Mean and standard deviation.*

	Pea size (PS)		Veraison (V)	
Site	Ψ_{PLWP} Predawn	Ψ_{MLWP} Midday	Ψ_{PLWP} Predawn	Ψ_{MLWP} Midday
1	-0.33 (0.04)	-1.29 (0.05)	-0.47 (0.12)	-1.38 (0.07)
2	-0.43 (0.08)	-1.21 (0.16)	-0.54 (0.13)	-1.44 (0.08)
3	-1.43 (0.01)	-1.48 (0.04)	-0.82 (0.21)	-1.76 (0.07)
4	-1.27 (0.04)	-1.39 (0.05)	-0.47 (0.05)	-1.58 (0.06)
5	-1.28 (0.03)	-1.50 (0.00)	-0.92 (0.08)	-1.50 (0.04)

Table 25. *Values for abscisic acid concentration (ABA) for sites 1, 2, 3, 4 and 5 at two different stages of growth -pea size (PS) and veraison (V)- at Predawn and Midday. Mean and standard deviation.*

	Pea Size (PS)		Veraison (V)	
Site	[ABA] Predawn	[ABA] Midday	[ABA] Predawn	[ABA] Midday
1	152.8 (4.7)	195.0 (33.4)	162.8 (7.6)	243.5 (13.1)
2	181.1 (21.4)	226.4 (5.9)	92.5 (8.7)	115.5 (3.6)
3	152.0 (17.3)	229.0 (42.2)	97.3 (15.5)	89.9 (8.6)
4	211.8 (5.5)	423.0 (80.7)	83.7 (2.4)	134.8 (38.7)
5	196.3 (5.9)	400.1 (19.8)	114.9 (12.7)	178.8 (9.3)

Table 26. *Analysis of the differences between groups using the non-parametric Kruskal-Wallis test. Pea Size (PS), veraison (V) and ripeness (RP).*

Conditions	Hour	Phenological stage	p-Value
Leaf Water Potential	Predawn	PS	0.014
	Midday	PS	0.014
	Midday	V	0.017
Abscisic acid content	Predawn	PS	0.019
	Midday	V	0.017
Leaf surface temperature	Predawn	PS	0.012
Total anthocyanins		Wine	0.019
Glycosylated Anthocyanins		Wine	0.014
Acetyl Glycosylated Anthocyanins		Wine	0.011
Berry Weight			0.009
Total Leaf Area /Kg		V	0.024
		RP	0.019

Table 27. *Pearson correlation matrix. Bold values are different from 0 at a significance level (α of 0.05. Abscisic acid (ABA), Pre down leaf water potential (ΨPLWP), midday leaf water potential (ΨMLWP), leaf surface temperature (Ts), total anthocyanins (ANT), glycosylated anthocyanins (A-G), acetyl glycosylated anthocyanins (A-AG), and total leaf area (TLA) around veraison (V), ripeness (RP), and pea size (PS).*

Pearson Correlation Matrix	[ABA] (ng/g) Predawn PS	[ABA] (ng/g) Midday V	Ψ_{PLWP} (MPa) Predawn PS	Ψ_{MLWP} (MPa) Midday PS	Ψ_{MLWP} (MPa) Midday V	Ts (°C) Predawn PS	Berry weight (g)	TLA/Kg V	TLA/Kg RP	ANT (mg/L) wine	A-G (mg/L) wine	A-AG (mg/L) wine
[ABA] Predawn PS	**1**											
[ABA] Midday V	-0.143	**1**										
Ψ Predawn PS	-0.283	0.489	**1**									
Ψ Midday PS	-0.011	0.145	**0.798**	**1**								
Ψ Midday V	0.116	**0.696**	**0.796**	**0.574**	**1**							
Ts Predawn PS	-0.226	0.451	**0.935**	**0.763**	**0.702**	**1**						
Berry weight	-0.228	0.316	**0.783**	**0.697**	0.492	**0.917**	**1**					
TLA/Kg V	**-0.516**	**0.627**	**0.738**	0.437	**0.578**	**0.703**	**0.566**	**1**				
TLA/Kg RP	-0.304	**0.682**	0.015	-0.262	0.190	-0.006	-0.161	0.478	**1**			
ANT - wine	0.319	**-0.703**	**-0.588**	-0.408	-0.461	**-0.737**	**-0.752**	**-0.699**	-0.451	**1**		
A-G - wine	0.278	**-0.680**	**-0.576**	-0.380	-0.461	**-0.752**	**-0.776**	**-0.661**	-0.407	**0.986**	**1**	
A-AG - wine	**0.610**	**-0.628**	**-0.658**	-0.411	-0.393	**-0.732**	**-0.738**	**-0.846**	-0.449	**0.917**	**0.882**	**1**

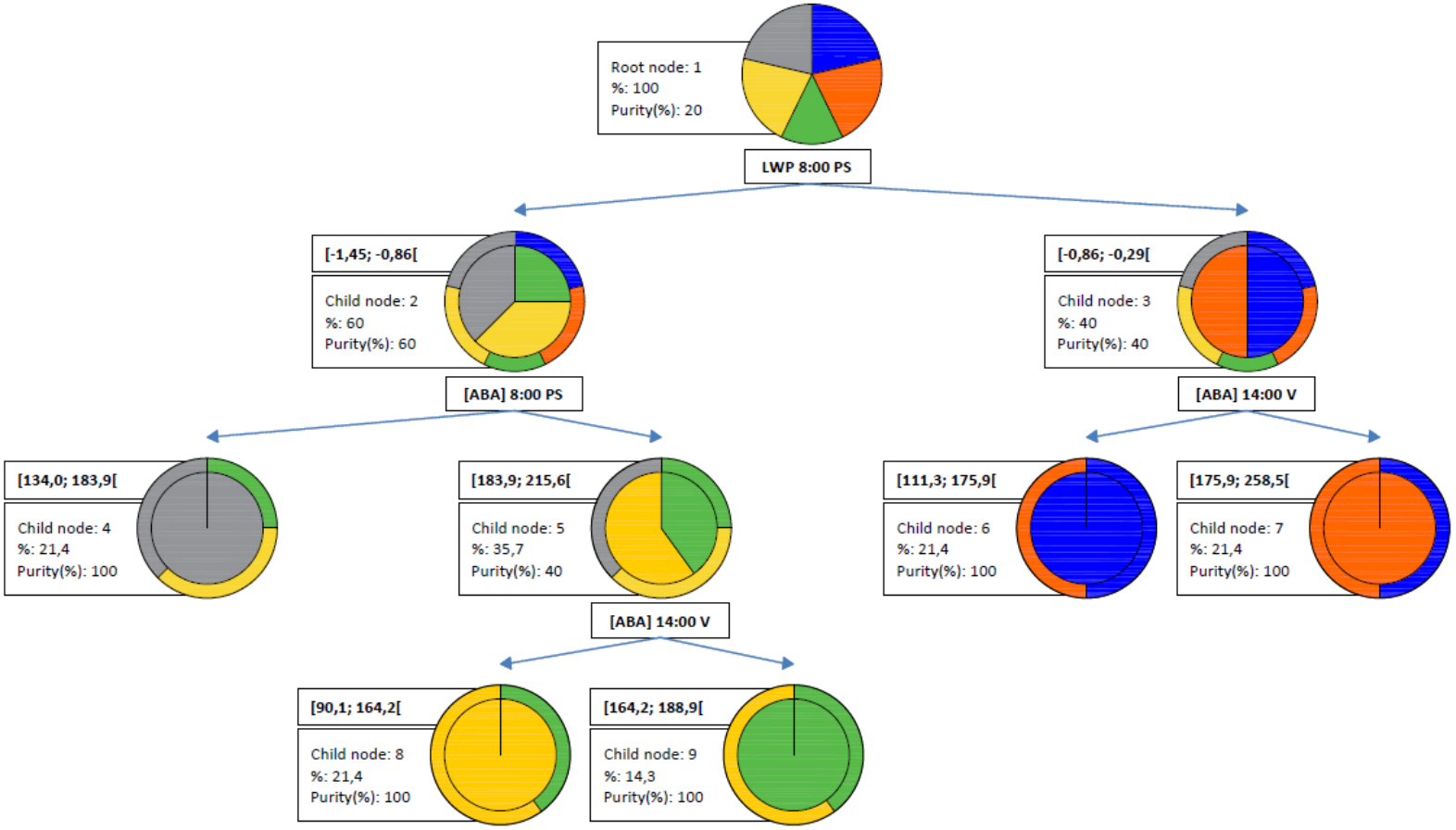

Figure 53. *Classification and Regression Trees by water indicators (LWP, ABA and Ts). Site 1 (red), Site 2 (blue), Site 3 (grey), Site 4 (orange) and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child notes are broken down to separate out of the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each colour represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the if-else question. The circles on the right branch correspond to those vineyards with higher values; those on the left to those with lower values, in answer to the if-else question (values are shown in brackets).*

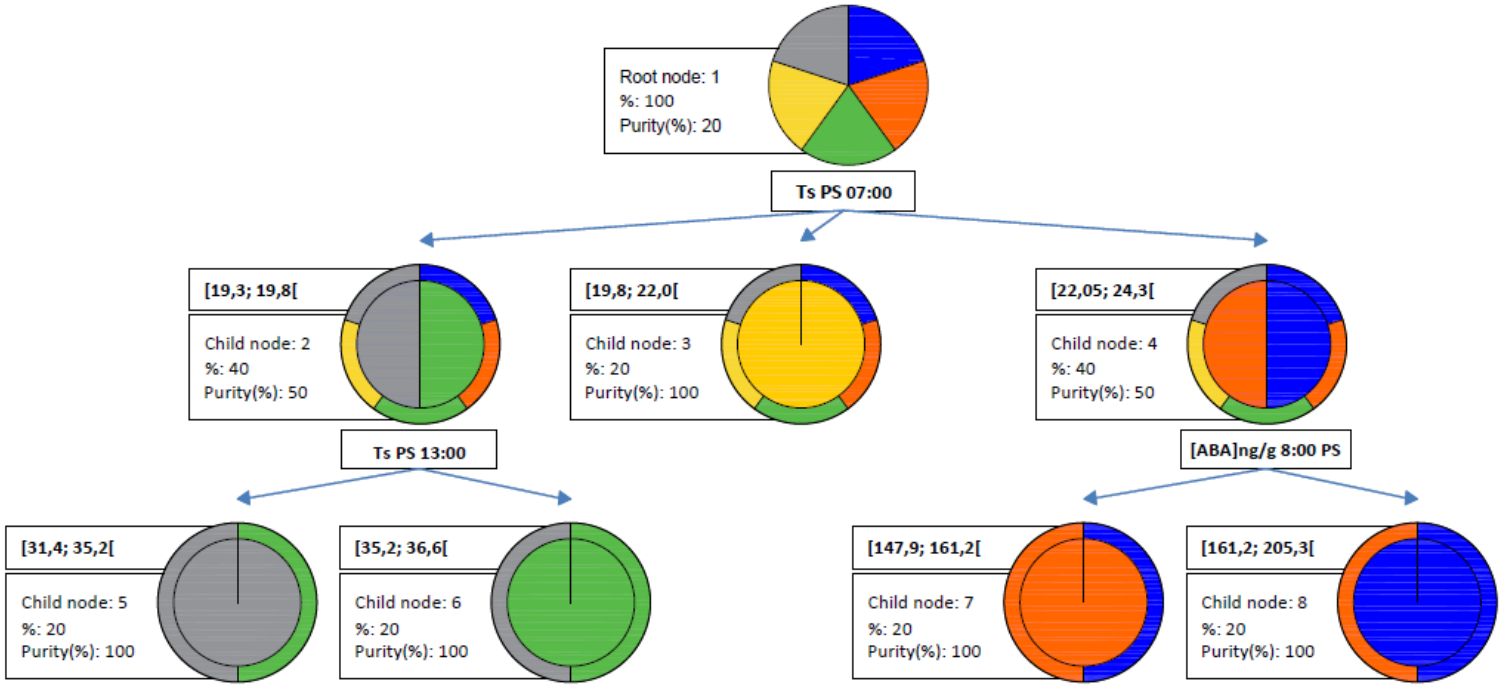

***Figure 54**. Classification and Regression Trees by Ts (surface canopy temperature). Site 1 (red), Site 2 (blue), Site 3 (grey), Site 4 (orange) and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child notes are broken down to separate out of the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each colour represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the if-else question. The circles on the right branch correspond to those vineyards with higher values; those on the left to those with lower values, in answer to the if-else question (values are shown in brackets).*

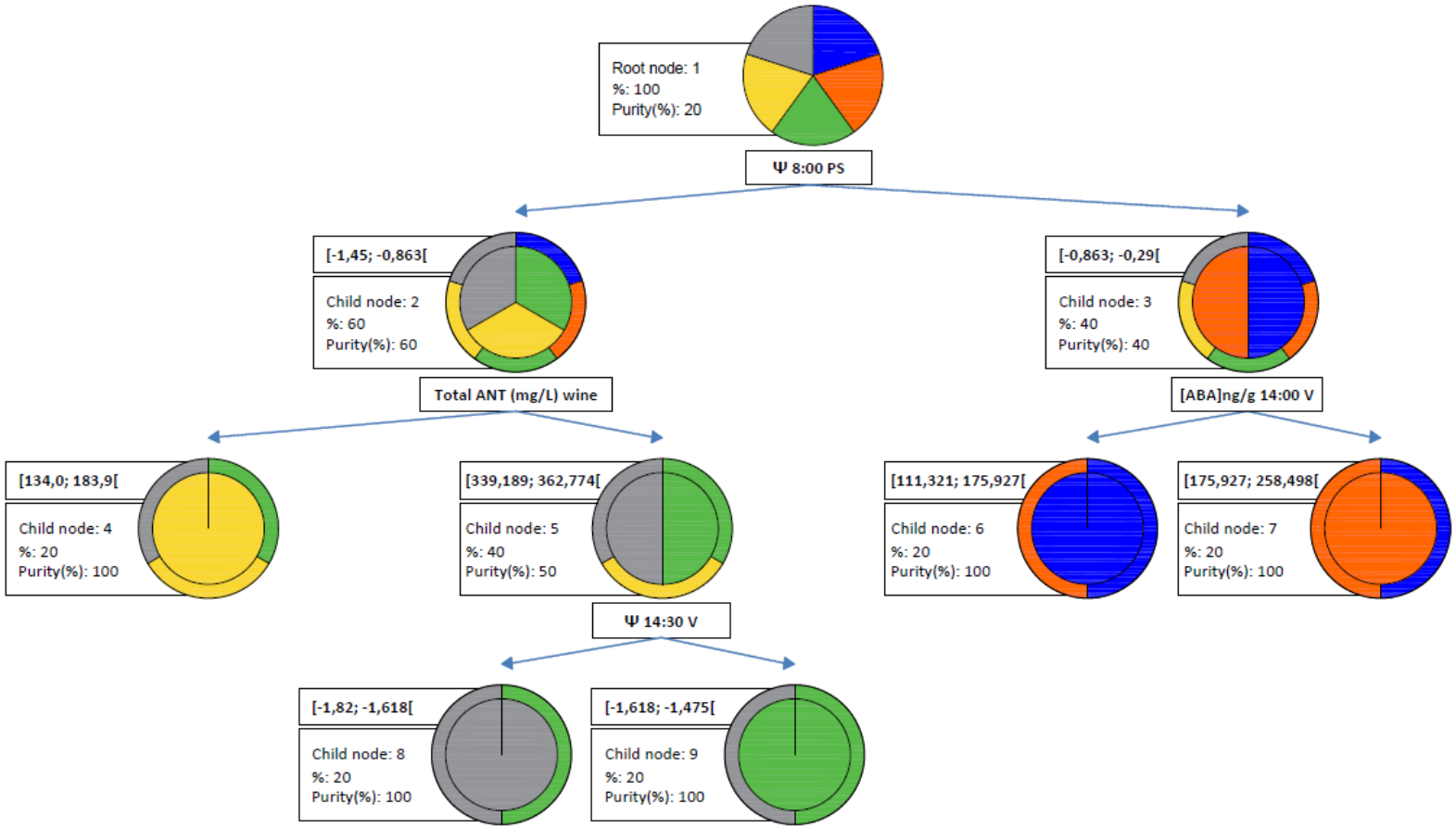

Figure 55. *Classification and Regression Trees by total anthocyanins. Site 1 (red), Site 2 (blue), Site 3 (grey), Site 4 (orange) and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child notes are broken down to separate out of the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each colour represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the if-else question. The circles on the right branch correspond to those vineyards with higher values; those on the left to those with lower values, in answer to the if-else question (values are shown in brackets).*

4.5 Conclusions

Regarding the results obtained from the characterization of Carignan, during warm years the highest phenological differences between early and late regions were recorded, which reached the maximum of a week at budbreak and veraison. The start of budbreak is delayed in years of low winter temperatures, but this delay does not seem to affect the variations in the harvest date. To highlight the effect of seasonal climate variability: the temperature rise in spring and autumn affects a shortening between phenological stages in the late region, causing advances in flowering and harvest. The warm autumn also has a noticeable effect on the elongation cycle of the vine in the early region, prolonging the period from harvest to leaf drop.

These abiotic factors causing vine stress notably higher in warmer areas than colder, lead us to consider the need of an improvement in the system of cultivation in each specific area (PO and EM). Adequate management in winemaking it should be adapted in the short and medium term, ensuring continued quality of the grapes and therefore the final product. However, the most restrictive in terms of water, bring to a new way of work trying to improve the efficiency of water use. In general, inter vineyards differences are much less accused during temperate vintage (2010) compared to warm and hot vintage (2009). Regards to vine production, soils with higher percentage of finer elements (Site 1, Site 5) had 20%-35% more yield than the stony plots. The soil texture directly influences on growth. The stony soil in Site 4 lead to diminishes the plant vigour and yield. However, the other two plots of PO (late ripening) are able to maintain the leaf area during summer. As regards to the wine, alcohol content and anthocyanin vary between plots and in the vintage. The total acidity appears to be associated with late mesoclimate (PO). Conversely, levels of tannins are still high in the early region (EM) and especially if the year is dry. Anthocyanin content in dry years is higher in late region (PO). The evolution of sugars and acids in berries follow a different pattern from that of the phenolic compounds. In years of high temperatures, the fruits reach a high degree of sugars, without having adequately ripened the skins and seeds, so the accumulation of phenolic compounds has not reached its maximum concentration.

To sum up, the climatic variability at the end of ripening is mainly determined by the increase in temperature and the deficit in vapor pressure. The persistence in the ripening season of high VPD values gives rise to notable inter-plot differences in warm years and early zone. In general, the plots with higher vegetative growth and lower production (lower Ravaz index and high leaf area/production ratio) would be more vulnerable to climate change, less predictable in relation to the composition of the grape and wine with variations in the composition of the grape in time of maturation. In warm years, grapes in the early zone reach a higher alcoholic degree than in the late zone, whereas the acidity is significantly higher in the late zone. Anthocyanins show variability in terms of vintage and

plot, whereas the accumulation of tannins is more notable in grapes from early and warm regions.

The climatic characterization of two completely different vintages and the choice of five plots located in two different ripening zones, has allowed us to establish ranges in terms of the polyphenolic composition in grapes and wine. The prediction of an interval of concentrations of anthocyanins and tannins is extremely important to define qualities and styles of wine, given the great inter-parcel variability observed in plots of old Carignan vineyards within the Priorat DOQ.

Regarding the results obtained from the characterization of Grenache, the EM plot, with a more northerly orientation, a poorer soil texture and more extreme climatic conditions of drought, would lead to lower vegetative growth, not always associated with greater water stress. On vineyards such as EM, having more available water in temperate years results in more balanced growth, meaning that the phenolic ripening of the grapes is more optimal.

During more temperate years (2010), a clear inter-plot difference is seen in the phenolic composition of the grapes (colour and tannins); in contrast to the warmer year (2011), the difference between plots is not so noticeable. This suggests that in the case of the cultivar Grenache, the composition of the grapes was clearly affected by the climate of the vintage, given that in warmer years, the composition of the grapes showed no difference. The concentration of anthocyanins in grapes between the two years is very similar. In contrast, the composition of total anthocyanins in wine in the warmest year exceeds the more temperate. A possible interpretation of this fact may be that Grenache has a good response in terms of adaptation capacity to warmer conditions; thus the difference in anthocyanin composition is more affected by the vintage than location of the vineyard, which would explain the higher concentration during 2011 and the smaller difference between plots in a warmer vintage.

Small berries from EM produced the highest levels of anthocyanins. EM always has the highest content in total anthocyanins (ANT T), extractable anthocyanins (ANT E), TPI and DMAC in both years. Concerning the wines, the highest concentration of anthocyanin were found in the EM treatment, with greater differences that LO in 2010. Grenache vines growing under warm climate conditions (Priorat DOQ), in heterogeneous-stony soils, showed notably variability in the wine composition in front of climate change.

It can be concluded that under more temperate climatic conditions, the maturation and polymerization of tannins has been slower in LO. Therefore, the quality of the wine parameters is more marked by the climate of the year than by the topographic situation of the study area, although the extraction in the wines can be modified by other factors such as winemaking techniques and alcohol content. Harvest takes place in 2 or 3 weeks of difference between the early and later areas. At the same time, the topography gives rise

to different exposures and different orientations, which makes the ripening of grapes to change within a single municipality.

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Chapter 5. Small-scale fermentation methodology to be used in vineyard heterogeneity

5.1 Chapter Summary

Researchers typically perform winemaking experiments using small amounts of grapes. Few studies have actually evaluated the effectiveness of small-scale fermentations – do they result in reliable and representative data for replication in larger-scale commercial production? Our research indicates that yes they do. Likewise, we have studied the pros and cons of employing different small-scale fermentation vessels. Fermentation vessel size should be carefully considered depending on the purpose of a proposed research. Some grape varietals skins release wine phenols more readily than others; when a sampling is not large enough to undergo a full-scale fermentation, such phenols might not fully extract into the wine. This study examines how the volumes (10, 25, 50 and 100L) can affect the composition of resulting wines and which is large enough to conclusively represent a specific winemaking procedure. Additionally, we carried out a commercial fermentation using a 2.500L vat. Tempranillo and Cabernet Sauvignon showed markedly different kinetics during fermentation. The medium size-vessel (25-50L) would give the best extraction for the phenolic composition of both wines. Taking into account which size of the smaller test vessels was employed, their patterns led to very good reproducibility for the ABV, pH and TA (CV≤ 7%) as well as ANT, TPI and tannins (CV≤ 20%). The HPLC phenolic composition also shows a low coefficient of variation (CV≤20%). This gives us the basis for validating small-scale fermentations applied to research studies.

5.2 Introduction

Research in viticulture relies mostly on measuring yield and grape composition to assess which management practices convey an improvement in vine performance and, as a consequence, could be worth implementing in the field (Ferreira *et al*., 2014). Although useful, this approach does not allow a completely satisfactory evaluation, since researchers cannot assess the extent to which the effects observed are transferred to the composition of the wine; i.e. to the quality of the final product (Pascual *et al*., 2016).

In order to overcome this limitation, some researchers introduce small-scale fermentations in their experiments to obtain a more complete evaluation, which is widely recognized as a positive step forward in the applicability of research (Sampaio et al., 2007). However, despite its relevance, little attention has been paid to evaluating the extent to which reducing grape processing volume in small-scale winemaking affects fermentation dynamics, wine composition, and reproducibility. On the one hand, authors such as Baker

et al. (1978) Mirabel et al. (1999), Dallas et al. (2003), González-Manzano et al.(2004) and Kroll et al. (1956) have published results addressing grape-seed extraction in model wine solutions, and others such as Lopes et al. (2002) and Rossouw et al. (2012) report on yeast performance as influenced by commercial and small-scale tanks. On the other hand, in a direct comparison of small-scale to commercial winemaking, Casalta et al. (2010) compared the aromatic compounds of Chardonnay, and noted that only three experiments used different fermentation volumes in red varieties. Schmid et al. (2007) compared three wine volumes (20, 50, and 300 kg) of a blend of Cabernet Sauvignon and Cabernet Franc, in an experiment that focused on evaluating the suitability of frozen must, and reported that winemaking outcomes were comparable among the three volumes compared. In the same research team, Jiranek (2010) and Schmid and Jiranek (2011) compared fresh, frozen, and blast-frozen grape fermentation using two different volumes (80 and 500 kg), and concluded that the wines were similar under wine tasting conditions. Finally, Sampaio et al. (2007) compared a small volume of Pinot Noir (3.5 kg) to a commercial fermentation (4540 kg), and observed that it was possible to effectively control oxidation and spoilage at this volume, although significant differences were observed in wine composition between both scales.

From this information, it can be seen that the existing research in this field is scarce. Therefore, while taking into account that small-scale winemaking conditions vary between experiments, as regional or winemaker preferences and protocol modifications may affect any stage of winemaking (i.e. yeast inoculation, cap management regime, and malolactic fermentation), there is a clear need to understand how conditions (particularly tank size) affect the composition of the wines obtained (Cerpa-Calderon and Kennedy, 2008). Moreover, the above-mentioned research did not consider repeatability, which is particularly relevant since high variability may limit potential buyer interest in purchasing from small-scale wineries, and an additional source of variation could interfere with data analysis.

The aim of this work was to evaluate the repeatability and reproducibility of small-scale winemaking. The differential aspect of this research was that four replicates were used with four different volumes of two distinct red varieties (Lasanta *et al*., 2014) Tempranillo and Cabernet Sauvignon, and that the small-scale fermentation protocols used mirrored typical winemaking techniques commonly used in small wineries producing premium red wines worldwide.

5.3 Materials and methods

5.3.1 Experimental design

This research was conducted in the experimental winery of the Enology Faculty in Tarragona, Spain, using grapes from the faculty experimental vineyards (41°8'54'' N, 1°11'54''E, Altitude: 50 m). The vineyards are located near the coast in the Designation of Origin Tarragona (Spain), which has a Mediterranean climate. The soils are typically fertile and dense, and are managed according to standard practices in the region. Grapes from two distinct varieties were used –Tempranillo (TE) and Cabernet Sauvignon (CS)– with the former based on large berries and low-to-medium phenolic potential, and the latter based on small sized berries and high phenolic content. For both varieties, four different small-scale volumes (10, 25, 50, and 100 L) were compared. All vessels had a ratio height/diameter ranging between 1.4 and 1.5. All tanks were made of stainless steel, with a rubber gasket to help keep the lid tight. For each variety and tank volume, four replicates were vinified. Additionally, a commercial-sized large fermentation was performed in a 2500 L stainless steel tank.

5.3.2 Grape analysis

All grape batches were analysed before they were introduced into each tank. One hundred berries from each variety were used to determine the sugar level, acidity, and pH, and another 300 berries were used to analyse phenolic maturity. Sugar content was determined using a handheld portable refractometer (Model 102/112/102bp). Titratable acidity (TA; g/L) was measured by titration with sodium hydroxide, and pH was measured using a pH meter (Crison Micro CM 2201). The modified Glories method, consisting of berry samples macerated at pH 3.6 instead of pH 3.2 (Nadal., 2010) was used to analyse phenolic maturity. Berries were blended (Oster Blender Classic 3 Model 4655) and macerated in an agitator (Edmund Bühler GmbH SM-30) to determine total anthocyanin (T Ant) and tannin content (Ribéreau-Gayon *et al*., 2003) (Ribéreau-Gayon and Stonestreet, 1965).

5.3.3 Wine analysis

Alcohol by volume (ABV), pH, TA, T Ant, and tannins within each tank size were analysed. Anthocyanin content was determined following the methodology detailed in Valls *et al.* (2009) and adapted from Deviliers et al. (2004) through high-performance liquid chromatography (HPLC) using a Hewlett Packard Liquid Chromatograph (Waters Corporation, Mildford, MA, USA) equipped with a Zorbax Eclipse Plus C18 Column (150 × 2.1 mm; 3.5 µm) and a Zorbax Eclipse Plus-C18 Precolumn (12.5 × 4.6 mm; 5 µm). Injection volume was 5 µL; elution was performed with a mobile phase A of HPLC-grade

water (0.2 % trifluoroacetic acid) and a mobile phase B using methanol (0.2 % trifluoroacetic acid). The column temperature was set at 50 ºC and the HPLC was coupled to a Diode Array Detector (DAD). Quantifications were performed using the DAD detector, and identifications were made considering the time of flight (TOF). A mass spectrometry (MS) detector was used to assist in the identification. Free anthocyanin content was determined using a calibration curve (based on peak area, $y = 0.7968x + 7.5756$; $R^2 = 0.9774$), which was established using malvidin 3-glucoside standard solutions submitted to the same procedure. Anthocyanidin-3-monoglucosides and respective acetylated and coumaroylated glycosides were identified on the basis of their ultraviolet–visible (UV–vis) spectra and retention times (**Table 28**). Anthocyanidins were identified by HPLC, making a comparison with internal standards. Calibration curves were obtained by injecting standards with different concentrations of malvidin 3-glucoside (Extrasynthese, Genay, France). The range of the linear calibration curves was 0.1 to 1.0 mg/L for the lower ($R^2 > 0.996$), 0.1–5.0 mg/L for intermediate ($R^2 > 0.987$), and 10.0–200.0 mg/L for the higher concentration compounds ($R^2 > 0.987$). Unknown concentrations were determined from the regression equations, and the results were expressed as milligrams of malvidin 3-glucoside. Repeatability of HPLC analysis gave a coefficient of variation of <7 %.

***Table 28**. Peak assignments, retention times, and mass spectral data of anthocyanidins.*

Peak #	Analytes	Retention	(m/z)	Code Id.
1	Delphinidin 3-O-glucoside	10.8	465	Dp3G
2	Cyanidin 3-O-glucoside	11.8	449	Cy3G
3	Petunidin 3-O-glucoside	12.5	479	Pt3G
4	Peonidin 3-O-glucoside	13.4	463	Pn3G
5	Malvidin 3-O-glucoside	13.8	493	Mv3G
6	Delphinidin 3-O-acetilglucoside	15.3	507	Dp3AG
7	Cyanidin 3-O-acetilglucoside	16.2	491	Cy3AG
8	Petunidin 3-O-acetilglucoside	16.7	521	Pt3AG
9	Peonidin 3-O-acetilglucoside	17.6	505	Pn3AG
10	Malvidin 3-O-acetilglucoside	17.8	535	Mv3AG
11	Delphinidin 3-O-cumarilglucoside	17.6	611	Dp3CG
12	Cyanidin 3-O-cumarilglucoside	18.5	595	Cy3CG
13	Petunidin 3-O-cumarilglucoside	18.7	625	Pt3CG
14	Peonidin 3-O-cumarilglucoside	19.3	609	Pn3CG
15	Malvidin 3-O-cumarilglucoside	19.4	639	Mv3CG

Code assignments: Dp3G (Delphinidin 3-O-glucoside), Cy3G (Cyanidin 3-O-glucoside), Pt3G (Petunidin 3-O-glucoside), Pn3G (Peonidin 3-O-glucoside), Mv3G (Malvidin 3-O-glucoside), Dp3AG (Delphinidin 3-O-acetilglucoside), Cy3AG (Cyanidin 3-O-acetilglucoside), Pt3AG (Petunidin 3-O-acetilglucoside), Pn3AG (Peonidin 3-O-acetilglucoside), Mv3AG (Malvidin 3-O-acetilglucoside), Dp3CG (Delphinidin 3-O-cumarilglucoside), Cy3CG (Cyanidin 3-O-cumarilglucoside), Pt3CG (Petunidin 3-O-cumarilglucoside), Pn3CG (Peonidin 3-O-cumarilglucoside), Mv3CG (Malvidin 3-O-cumarilglucoside).

Procyanidins were analysed by injecting 3 µl of wine samples through Rapid Resolution Liquid Chromatography (RRLC) using a Zorbax Eclipse XDB-C18 (50 × 30; 1.8 µm)

followed by a RRLC in-line pre-column (4.6 mm, 0.2 µm) at 30 °C. The HPLC injection volume was 1.4 µL, with a 0.7 mL/min flux; mobile phase A: water (0.1 % formic acid), mobile phase B: methanol (0.1 % formic acid). Phenolic compounds were identified according to their order of elution, retention times of pure compounds (gallic acid, catechin, procyanidin dimer B2, mono gallate dimer, procyanidin trimer C1, and epicatechin gallate) and their molecular masses. **Table 29** shows the retention time and *m/z* for each compound.

***Table 29**. Peak assignments, retention times, and mass spectral data of procyanidins.*

Peak #	Analytes	Retention	(m/z)	Code Id.
1	Procyanidin trimer C	0.6	865.1989	ptC
2	Gallic acid	0.8	169.0147	GA
3	Procyanidin dimer B3	1.9	577.1364	pdB3
4	Procyanidin dimer B1	2.1	577.1364	pdB1
5	Procyanidin trimer T2	2.4	865.1989	ptT2
6	(+)-Catechin	2.8	289.0722	Cat
7	Procyanidin dimer B4	3.4	577.1364	pdB4
8	Procyanidin dimer B2	3.7	577.1364	pdB2
9	Procyanidin dimer B2-3-O-gallate (Dimer monogallate)	4.5	729.1469	PdB2MG1
10	Procyanidin dimer B2-3′-O-gallate (Dimer monogallate)	4.7	729.1469	PdB2MG2
11	(-)-Epicatechin	5.0	289.0722	EC
12	Procyanidin trimer C1 (-)-epicatechin-3-O-gallate	5.0	865.1989	ptECG
13	Procyanidin dimer B1-3-O-gallate	5.1	577.1364	pdB1G1
14	Dimer digallate	5.7	881.1683	DDG
15	(-)-Epicatechin-O-gallate	6.2	441.0835	ECG
16	Procyanidin dimer B1-3′-O-gallate	6.6	577.1364	pdB1G2

Code assignments: ptC (Procyanidin trimer C), GA(Gallic acid), pdB3 (Procyanidin dimer B3), pdB1 (Procyanidin dimer B1), ptT2 (Procyanidin trimer T2), Cat ((+)-Catechin), pdB4 (Procyanidin dimer B4), pdB2 (Procyanidin dimer B2), PdB2MG1(Procyanidin dimer B2-3-O-gallate), PdB2MG2 (Procyanidin dimer B2-3′-O-gallate), EC ((-)-Epicatechin), ptECG (Procyanidin trimer C1 (-)-epicatechin-3-O-gallate), pdB1G1 (Procyanidin dimer B1-3-O-gallate), DDG (Dimer digallate), ECG ((-)-Epicatechin-O-gallate), pdB1G2 (Procyanidin dimer B1-3′-O-gallate).

5.4 Results

5.4.1 Grape composition

Grape composition before fermentation was very similar for all tank sizes (**Table 30**), and low variability occurred between tanks of the same size (coefficients of variation [CV] <5 %). This finding was essential, to guarantee that the differences eventually observed in wine composition were not due to differences in grape composition, but were associated with the winemaking process.

***Table 30**. Must composition and berry weight of each tank. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Volume	Brix	pH	TA (g/L)	Bw (g)
TE-10	23.1±0.1[a]	3.42±0.02[a]	6.46±0.12[a]	2.21±0.11[a]
TE-25	23.1±0.1[a]	3.40±0.01[ab]	6.53±0.15[a]	2.30±0.14[a]
TE-50	23.2±0.1[a]	3.44±0.02[b]	6.60±0.10[a]	2.20±0.13[a]
TE-100	23.2±0.1[a]	3.41±0.01[a]	6.54±0.11[a]	2.33±0.08[a]
CS-10	23.8±0.1[b]	3.26±0.02[b]	5.00±0.10[a]	1.39±0.14[b]
CS-25	24.1±0.1[a]	3.21±0.01[c]	5.20±0.10[a]	1.43±0.06[b]
CS-50	24.1±0.1[a]	3.26±0.02[b]	5.18±0.07[a]	1.51±0.12[b]
CS-100	23.8±0.1[b]	3.26±0.01[b]	5.21±0.06[a]	1.56±0.12[b]

TA: titratable acidity. Bw: berry weight.

5.4.2 Fermentation performance

Winemaking conditions allowed adequate fermentation dynamics in the 32 tanks included in the experiment, achieving a complete transformation of sugars into ethanol.
Density rapidly decreased after the second day of fermentation for both varieties, and 5 and 9 days after the start of fermentation, with only a small quantity of sugar (ρ = 1010 kg/m^3) remaining in TE and CS, respectively. At this point, the second stage of fermentation (slow fermentation process) began and, after 3 days, the remaining sugars were transformed into alcohol.

In general terms, the complete alcoholic fermentation of TE and CS could be divided into two different stages: tumultuous and slow. The duration of tumultuous fermentation varied according to the composition of the must and the temperature at which it was carried out. Grapes were stored at 21 °C in a cooler before crushing. The cellar temperature was set at 22 °C and the temperature in the tank was held at 28 °C at the tumultuous stage to ensure good extraction of polyphenols. This step should be carefully considered to avoid uncontrolled fermentation and make this methodology reliable. The yeasts developed comfortably, thus ensuring the total transformation of all sugar into alcohol for both grape varieties. Density rapidly decreased after the second day of fermentation for each variety and vessel (**Figure 56**).

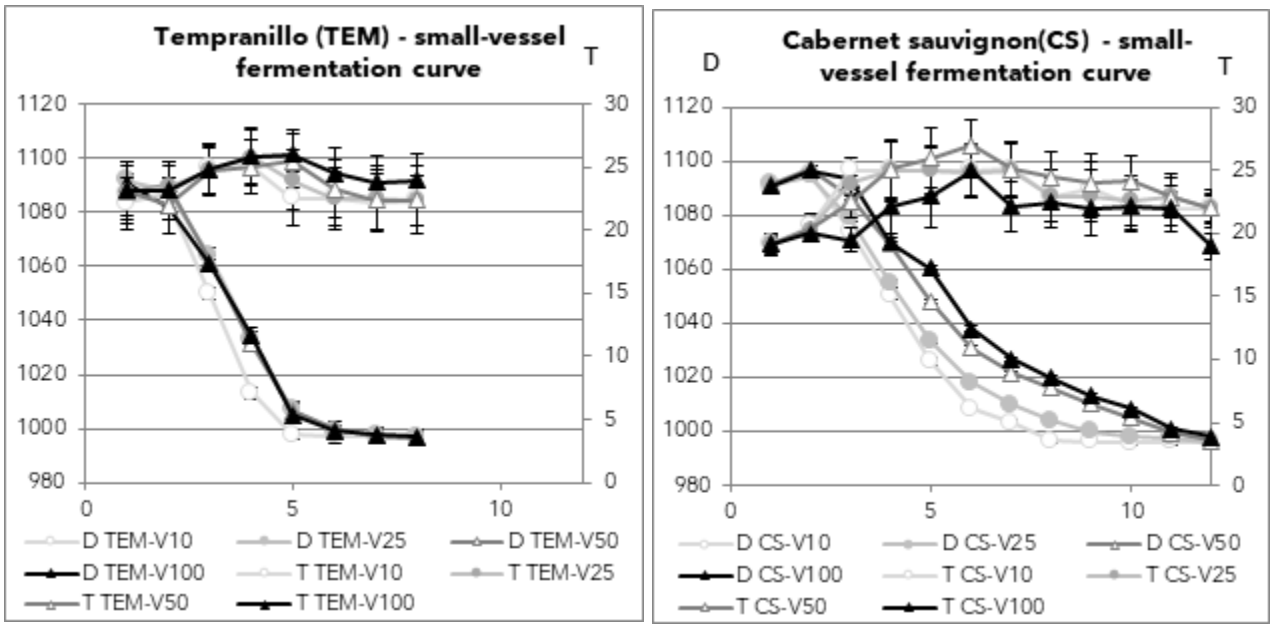

Figure 56. *Evolution of fermentation in small-vessels. Evolution of density (D) and temperature (T) during fermentation.*

Approximately 6 and 10 days after the start of fermentation (for TE and CS, respectively), a small quantity of sugar corresponding to a density of ρ = 995 kg/m^3 remained in the must. At this point, the last stage of fermentation transformed the remaining final grams of sugar into alcohol over the following 3-4 days. TE showed a rapid decrease until the 5th day of fermentation, when it reached ρ = 997.8, 1007.0, 1006.5, and 1005.0 kg/m^3, respectively, for each increasing small-scale volume (25, 50, 75, and 100 L). Fermentation kinetics in TE required 8 days to ferment all the reducing sugars, showing a slow decrease for the last 3 days. The CS required 12 days to complete the fermentation process. Temperatures did not exceed 28 °C for both kinetics under the same conditions of controlled room temperature and vessel size. After fermentation, the temperature decreased to 22 °C in both cases.

Modelling data using linear functions proved easier for predicting the kinetics of the fermentation processes of both varieties/volume studies. As tumultuous fermentation occurred with a different duration for each variety compared with the slow stage, two regression curves were calculated for each combination variety/volume. As expected, in the tumultuous phase (when maximal fermentation activity occurred) and slow fermentation stage (after tumultuous fermentation), two slopes were clearly distinguished on the fermentation curves for both varieties (**Table 31**). Linear regression slopes of the tumultuous stage ranged between −21.933 and −24.850 for TE and −12.286 and −17.321 for CS, indicating faster kinetics for TE in the tumultuous fermentation stage. The coefficient of determination was also higher in the tumultuous stage. Next, considering all volume vessels, the TE slopes from the tumultuous stage did not indicate substantially different kinetics between volumes, although for the 10 L capacity vessel, it appeared to

decrease faster, with a curve described by y = −24.8x + 1121, compared with the 25, 50, and 100 L vessels (y = −22.4x + 1124, y = −22.1x + 1122, and y = −21.9x + 1120, respectively), showing very similar slopes. However, the slow stage revealed a similar tendency, having the lowest slope for the 10 L vessel. CS showed a proportional relationship between slope and volume. The 10 L tank had the highest slope in the tumultuous stage (y = −17.3x + 1119), and the lowest slope on the slow stage (y = −1.9x + 1016), indicating that the tumultuous part of fermentation proceeded faster in the 10 L vessel than any other vessel evaluated.

***Table 31**. Kinetics of fermentation, tumultuous and slow stages.*

Treatment	Tumultuous fermentation stage	R^2 value	Slow fermentation stage	R^2 value
TEM 10L	y = −24.8x + 1121	0.95	y = −0.25x + 999	0.83
TEM 25L	y = −22.4x + 1124	0.95	y = −3.15x + 1020	0.76
TEM 50L	y = −22.1x + 1121	0.93	y = −3.2x + 1021	0.85
TEM 100L	y = −21.9x + 1120	0.97	y = −2.53x + 1016	0.80
CS 10L	y = −17.3x + 1119	0.95	y = −1.90x + 1016	0.68
CS 25L	y = −15.9x + 1118	0.96	y = −3.41x + 1034	0.85
CS 50L	y = −13.6x + 1118	0.93	y = −5.66x + 1062	0.98
CS 100L	y = −12.3x + 1117	0.90	y = −6.53x + 1074	0.98

5.4.3 Effect of small-scale tank volume on wine composition

With regards to the basic parameters of wine composition, tank size was observed not to influence ABV, pH, or TA in either CS or TE (**Table 32**), but it did affect phenolic composition (T Ant, and tannins). The highest T Ant values were observed in the intermediate sizes (25 and 50 L), whereas for tannin content, the highest values were found in the larger tanks (50 and 100 L) in both varieties.

***Table 32**. Wine analysis of tanks after fermentation of TE (Tempranillo) and CS (Cabernet Sauvignon). [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Volume	ABV	pH	TA (g/L)	T Ant (mg/L)	Tannins (g/L)
TE-10	12.82±0.05^{b}	3.63±0.01^{b}	5.25±0.12^{c}	311±7^{c}	2.3±0.2^{bc}
TE-25	12.90±0.08^{ab}	3.68±0.01^{b}	5.32±0.28^{bc}	367±15^{a}	1.9±0.5^{bc}
TE-50	12.77±0.03^{b}	3.75±0.03^{a}	5.54±0.24^{b}	385±14^{a}	2.7±0.5^{ab}
TE-100	12.91±0.02^{a}	3.76±0.01^{a}	5.60±0.05^{b}	342±7^{b}	3.0±0.1^{a}
CS-10	13.18±0.06^{a}	3.51±0.09	5.97±0.22^{ab}	341±20^{c}	1.3±0.6^{bd}
CS-25	13.23±0.06^{a}	3.57±0.04	6.00±0.12^{ab}	402±26^{a}	1.1±0.3^{cd}
CS-50	13.18±0.07^{a}	3.55±0.01	6.14±0.21^{ab}	379±23^{ab}	1.9±0.3^{b}
CS-100	13.25±0.04^{a}	3.53±0.01	6.27±0.14^{a}	363±25^{bc}	2.0±0.2^{b}

ABV: Alcohol by volume. pH: Potential hydrogen; TA: Titratable acidity in tartaric. T Ant: Total anthocyanin and tannins. Results show the mean value and standard deviation.

One of the most relevant effects of tank size from a research perspective is increasing or decreasing the variability of the composition of the wine obtained from replicates. When the CV obtained for each variable, tank size, and variety were compared, all values were

low, especially for ABV, pH, and TA (CV <4 %), but also for T Ant and tannin content (CV <8 %). Taking into account that the observed CVs were satisfactory for all tank sizes and varieties (less than 5 %), there was a slightly greater variability in the intermediate sizes (25 and 50 L) with TE. This observation supported the repeatability of wine quality at any of the tank sizes with regards to the major wine composition parameters.

***Table 33**. Anthocyanin wine profile (glucoside, acetyl glucoside and coumaroyl glucoside) for Tempranillo. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Analytes	C-2500L	10L	25L	50L	100L
Mv3G	70.3±8.1^{ab}	70.9±12.2^{ab}	84.5±8.4^{a}	85.1±13.4^{a}	60.8±2.3^{b}
Pt3G	8.4±1.8^{c}	15.1±2.6^{ab}	17.6±2.1^{a}	18.5±4.0^{a}	13.2±1.0^{b}
Dp3G	5.6±0.4^{a}	6.3±1.3^{a}	7.3±1.0^{a}	8.1±2.2^{a}	5.8±0.6^{a}
Pn3G	11.0±0.5^{b}	12.7±1.5^{a}	14.2±0.9^{a}	16.1±1.7^{a}	12.0±0.6^{b}
Cy3G	0.8±0.2^{a}	0.8±0.1^{a}	0.9±0.2^{a}	1.2±0.2^{a}	0.8±0.1^{a}
Total G	**96.1±1.0^{b}**	**105.9±17.7^{ab}**	**124.4±12.5^{a}**	**129.0±21.4^{a}**	**92.7±4.5^{b}**
Mv3AG	30.1±0.5^{b}	29.4±1.7^{b}	38.9±2.2^{a}	40.2±5.1^{a}	26.8±0.5^{c}
Pt3AG	0.9±0.2^{a}	1.0±0.2^{a}	1.4±0.2^{a}	1.5±0.3^{a}	0.9±0.0^{a}
Dp3AG	0.2±0.0^{a}	0.2±0.0^{a}	0.3±0.0^{a}	0.3±0.1^{a}	0.2±0.0^{a}
Pn3AG	2.9±0.2^{b}	2.8±0.3^{b}	3.9±0.3^{a}	4.1±0.5^{a}	2.6±0.2^{b}
Cy3AG	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}
Total AG	**33.7 ± 0.6 ^{b}**	**33.5 ± 2.2 ^{b}**	**44.7 ± 2.7 ^{a}**	**46.2 ± 6.1 ^{a}**	**30.5 ± 0.7 ^{b}**
Mv3CG	25.8±0.9^{ab}	27.6±2.6^{ab}	29.6±2.0^{a}	28.9±4.6^{a}	21.6±0.9^{b}
Pt3CG	4.1±0.4^{b}	4.9±0.9^{ab}	5.8±0.7^{a}	5.8±1.6^{ab}	3.6±0.4^{b}
Dp3CG	1.8±0.2^{b}	1.5±0.4^{b}	1.8±0.2^{ab}	1.8±0.6^{b}	1.2±0.2^{b}
Pn3CG	5.7±0.2^{ab}	5.1±0.5^{a}	6.2±0.4^{a}	6.6±1.3^{a}	4.2±0.2^{b}
Cy3CG	1.2±0.1^{b}	1.6±0.3^{a}	1.7±0.2^{a}	1.9±0.4^{a}	1.2±0.1^{b}
Total CG	**38.6±1.9^{ab}**	**40.7±4.7^{a}**	**45.1±3.5^{a}**	**44.9±8.6^{a}**	**31.8±1.8^{b}**

Dp3G (Delphinidin 3-O-glucoside), Cy3G (Cyanidin 3-O-glucoside), Pt3G (Petunidin 3-O-glucoside), Pn3G (Peonidin 3-O-glucoside), Mv3G (Malvidin 3-O-glucoside), Dp3AG (Delphinidin 3-O-acetilglucoside), Cy3AG (Cyanidin 3-O-acetilglucoside), Pt3AG (Petunidin 3-O-acetilglucoside), Pn3AG (Peonidin 3-O-acetilglucoside), Mv3AG (Malvidin 3-O-acetilglucoside), Dp3CG (Delphinidin 3-O-cumarilglucoside), Cy3CG (Cyanidin 3-O-cumarilglucoside), Pt3CG (Petunidin 3-O-cumarilglucoside), Pn3CG (Peonidin 3-O-cumarilglucoside), Mv3CG (Malvidin 3-O-cumarilglucoside).

T Ant composition (**Table 33** and **Table 34**) in the medium-sized tanks (25 and 50 L) was higher than any other volumes (10 and 100 L) in TE. Malvidin glucosides (G) were more highly extracted (up to one-third) than acetyl glucosides (AG). Furthermore, the latter showed almost the same concentration of coumaroyl glucosides (GC). In CS, the greatest anthocyanin contents were found in the biggest volumes (**Table 34**). CS tanks measuring 10 and 25 L showed delayed extraction of anthocyanins, giving 117.7 mg/L of T Ant in 10 L, 128.5 mg/L in 25 L, 361.9 mg/L in 100 L, and 384.4 mg/L in 50 L. Thus, in the case of CS, it appears that the larger the tank, the greater the extraction (50, 100). In CS, the difference between G and AG total concentration was not remarkable, with lower extractions observed in the smaller volumes in all cases. Reproducibility in terms of anthocyanin content can be said to be satisfactory, since the CVs for all anthocyanin families were below

20 %, with the median CV being 13 % for CS and 10 % for TE (**Figure 57**). Tank size appeared to affect reproducibility, although the observed effect was different for each variety. In TE, the lowest CVs were found for the 100 L and 25 L tanks, whereas in CS this occurred in the 10 L and 50 L tanks.

***Table 34**. Anthocyanin wine profile (glucoside, acetyl glucoside and coumaroyl glucoside) for Cabernet Sauvignon. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Analytes	C-2500 L	10L	25L	50L	100L
Mv3G	95.6±13.7^{a}	31.3±3.9^{b}	34.1±5.2^{b}	114.1±3.8^{a}	103.6±19.4^{a}
Pt3G	6.7±1.5^{a}	1.0±0.1^{b}	1.1±0.2^{b}	7.8±0.2^{a}	7.4±1.8^{a}
Dp3G	2.4±0.9^{a}	0.2±0.1^{b}	0.3±0.1^{b}	2.7±0.3^{a}	2.6±0.9^{a}
Pn3G	7.1±0.7^{ab}	8.2±1.6^{ab}	9.7±0.9^{b}	8.2±0.8^{ab}	7.7±0.7^{a}
Cy3G	0.1±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}
Total G	111.9±16.8^{a}	40.6±5.7^{b}	45.3±6.4^{b}	132.9±5.1^{a}	121.4±22.7^{a}
Mv3AG	173.0±15.4^{a}	69.2±7.7^{b}	74.4±7.9^{b}	209.7±10.2^{a}	200.7±20.4^{a}
Pt3AG	3.5±0.7^{a}	0.5±0.1^{b}	0.5±0.1^{b}	4.5±0.2^{a}	4.4±0.7^{a}
Dp3AG	0.8±0.2^{a}	0.1±0.0^{b}	0.1±0.0^{b}	0.9±0.1^{a}	0.8±0.2^{a}
Pn3AG	1.4±0.3^{a}	1.9±0.4^{a}	1.9±0.2^{a}	1.8±0.3^{a}	1.8±0.1^{a}
Cy3AG	0.2±0.0^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.2±0.0^{a}	0.2±0.0^{a}
Total AG	178.9±15.5^{a}	71.6±8.2^{b}	76.9±8.2^{b}	217.0±10.9^{a}	208.0±21.4^{a}
Mv3CG	22.3±4.6^{a}	4.0±0.7^{b}	4.8±0.5^{b}	30.5±1.7^{a}	28.2±4.6^{a}
Pt3CG	0.8±0.3^{a}	0.0±0.0^{b}	0.1±0.0^{b}	0.9±0.1^{a}	0.9±0.3^{a}
Dp3CG	0.1±0.0^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.1±0.0^{a}	0.1±0.0^{a}
Pn3CG	3.1±0.7^{a}	1.4±0.3^{b}	1.5±0.2^{b}	3.1±0.1^{a}	3.3±0.7^{a}
Cy3CG	0.1±0.0^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.1±0.0^{a}	0.1±0.0^{a}
Total CG	26.4±5.7^{a}	5.5±1.0^{b}	6.3±0.7^{b}	34.6±1.9^{a}	32.5±5.7^{a}

Description: Dp3G (Delphinidin 3-O-glucoside), Cy3G (Cyanidin 3-O-glucoside), Pt3G (Petunidin 3-O-glucoside), Pn3G (Peonidin 3-O-glucoside), Mv3G (Malvidin 3-O-glucoside), Dp3AG (Delphinidin 3-O-acetilglucoside), Cy3AG (Cyanidin 3-O-acetilglucoside), Pt3AG (Petunidin 3-O-acetilglucoside), Pn3AG (Peonidin 3-O-acetilglucoside), Mv3AG (Malvidin 3-O-acetilglucoside), Dp3CG (Delphinidin 3-O-cumarilglucoside), Cy3CG (Cyanidin 3-O-cumarilglucoside), Pt3CG (Petunidin 3-O-cumarilglucoside), Pn3CG (Peonidin 3-O-cumarilglucoside), Mv3CG (Malvidin 3-O-cumarilglucoside).

***Table 35**. Procyanidin wine profile (M: monomers, D: dimers and T: trimers) for Tempranillo. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Analytes	**C-2500L**	**10L**	**25L**	**50L**	**100L**
Gallic acid	41.0±0.1^{a}	21.1±0.9^{c}	19.5±1.0^{c}	23.6±2.9^{bc}	26.7±2.0^{b}
Cat	40.7±1.3^{a}	14.7±1.0^{bc}	13.0±0.7^{c}	17.7±2.3^{bc}	17.3±1.2^{b}
EC	15.0±0.4^{a}	10.1±0.8^{b}	9.5±0.5^{b}	13.2±1.7^{a}	12.8±0.8^{a}
ECG	0.5±0.0^{c}	0.8±0.1^{c}	1.4±0.1^{a}	1.5±0.1^{a}	1.1±0.1^{b}
Total M	97.3±1.8^{a}	46.8±2.8^{c}	43.2±2.3^{c}	56.0±7.0^{b}	57.9±4.0^{b}
pdB1	20.5±0.2^{a}	11.9±0.7^{c}	10.8±0.4^{c}	13.5±1.7^{bc}	13.3±0.8^{b}
pdB2	5.6±0.3^{d}	14.1±1.0^{b}	12.7±1.0^{c}	15.9±2.0^{ab}	16.8±0.9^{a}
pdB3	7.1±0.1^{d}	12.0±0.7^{b}	10.8±0.5^{c}	13.5±1.7^{ab}	13.3±0.7^{a}
pdB4	17.7±0.5^{a}	13.7±0.8^{b}	12.8±0.9^{c}	15.6±1.9^{ab}	16.2±1.0^{a}
pdB2MG1	5.5±4.1^{a}	1.2±0.2^{c}	1.3±0.3^{c}	1.9±0.4^{b}	1.6±0.2^{b}
pdB1G1	2.8±0.1^{b}	4.4±0.3^{a}	4.3±0.5^{a}	4.5±0.4^{a}	4.5±0.3^{a}
DDG	0.5±0.1^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
pdB1G2	5.4±0.2^{b}	6.6±0.8^{b}	10.4±6.4^{a}	7.1±0.5^{b}	6.9±0.4^{b}
Total D	65.0±5.6^{a}	63.9±4.6^{a}	63.1±10.2^{a}	72.0±8.6^{a}	72.5±4.3^{a}
ptC	5.0±0.0^{b}	21.7±0.9^{a}	21.8±1.7^{a}	22.9±2.0^{a}	22.6±1.1^{a}
ptT2	20.1±1.1^{a}	22.0±1.0^{a}	21.8±1.0^{a}	23.8±2.0^{a}	22.2±1.0^{a}
ptECG	15.7±0.6^{a}	11.6±1.1^{b}	9.7±0.5^{c}	12.1±1.4^{b}	12.4±0.9^{b}
Total T	40.8±1.7^{b}	55.3±3.0^{a}	53.4±3.2^{a}	58.8±5.4^{a}	57.2±3.0^{a}

Description: ptC (Procyanidin trimer C), GA(Gallic acid), pdB3 (Procyanidin dimer B3), pdB1 (Procyanidin dimer B1), ptT2 (Procyanidin trimer T2), Cat ((+)-Catechin), pdB4 (Procyanidin dimer B4), pdB2 (Procyanidin dimer B2), PdB2MG1(Procyanidin dimer B2-3-O-gallate), PdB2MG2 (Procyanidin dimer B2-3′-O-gallate), EC ((-)-Epicatechin), ptECG(Procyanidin trimer C1 (-)-epicatechin-3-O-gallate), pdB1G1 (Procyanidin dimer B1-3-O-gallate), DDG (Dimer digallate), ECG ((-)-Epicatechin-O-gallate), pdB1G2 (Procyanidin dimer B1-3′-O-gallate.

***Table 36**. Procyanidin wine profile (M: monomers, D: dimers and T: trimers) for Cabernet Sauvignon. [a]Values with different letters denote a statistically ($p<0.05$) significant difference. Mean and standard deviation.*

Analytes	C-2500L	10L	25L	50L	100L
Gallic	34.3±0.1^{a}	21.6±2.4^{c}	31.9±5.6^{a}	29.6±1.2^{a}	25.7±0.9^{b}
Cat	64.1±0.4^{a}	21.5±2.0^{d}	37.0±8.6^{b}	33.6±3.0^{bc}	29.6±1.3^{c}
EC	35.8±0.3^{a}	22.3±2.7^{b}	33.0±6.4^{a}	31.2±3.5^{a}	26.0±1.1^{b}
ECG	0.1±0.0^{c}	0.6±0.1^{b}	0.9±0.1^{a}	1.2±0.2^{a}	0.8±0.2^{a}
Total M	134.3±0.9^{a}	65.9±7.2^{c}	102.8±20.6^{b}	95.7±7.9^{b}	82.1±3.5^{b}
pdB1	20.7±0.6^{a}	11.0±0.7^{c}	15.8±3.4^{b}	14.9±0.7^{b}	13.5±0.6^{b}
pdB2	11.3±0.7^{c}	22.5±2.5^{b}	31.2±7.2^{a}	29.3±2.4^{a}	24.3±0.8^{b}
pdB3	8.7±0.6^{c}	11.1±0.9^{b}	15.6±3.3^{a}	14.9±0.5^{a}	13.5±0.5^{b}
pdB4	19.7±1.9^{b}	22.2±2.1^{b}	31.0±7.3^{a}	28.3±1.5^{a}	23.9±0.7^{b}
pdB2MG1	1.1±0.1^{c}	3.6±0.3^{a}	3.1±0.8^{b}	3.0±0.2^{b}	2.6±0.3^{b}
pdB1G1	2.4±0.1^{c}	3.7±0.4^{b}	4.8±0.9^{a}	4.7±0.3^{a}	4.1±0.2^{b}
DDG	0.4±0.1^{a}	0.3±0.0^{a}	0.2±0.1^{ab}	0.2±0.1^{ab}	0.2±0.0^{b}
pdB1G2	9.8±0.3^{b}	11.0±0.3^{b}	15.3±8.0^{a}	10.8±11.1^{b}	16.9±5.8^{a}
Total D	74.0±4.3^{b}	85.5±7.2^{b}	116.9±31.0^{a}	106.2±16.8^{a}	98.8±9.0^{a}
ptC	4.7±0.1^{c}	15.8±1.2^{b}	20.1±3.5^{ab}	19.1±1.0^{a}	14.6±8.4^{ab}
ptT2	15.7±2.1^{b}	15.3±1.4^{b}	22.0±4.7^{ab}	19.7±1.2^{a}	18.3±0.7^{ab}
ptECG	17.4±1.6^{b}	15.9±2.3^{ab}	22.1±4.7^{a}	19.9±1.4^{a}	16.8±0.6^{b}
Total T	37.8±3.8^{b}	47.0±5.0^{a}	64.2±12.9^{a}	58.8±3.6^{a}	49.7±9.8^{a}

Description: ptC (Procyanidin trimer C), GA(Gallic acid), pdB3 (Procyanidin dimer B3), pdB1 (Procyanidin dimer B1), ptT2 (Procyanidin trimer T2), Cat ((+)-Catechin), pdB4 (Procyanidin dimer B4), pdB2 (Procyanidin dimer B2), PdB2MG1(Procyanidin dimer B2-3-O-gallate), PdB2MG2 (Procyanidin dimer B2-3'-O-gallate), EC ((-)-Epicatechin), ptECG (Procyanidin trimer C1 (-)-epicatechin-3-O-gallate), pdB1G1 (Procyanidin dimer B1-3-O-gallate), DDG (Dimer digallate), ECG ((-)-Epicatechin-O-gallate), pdB1G2 (Procyanidin dimer B1-3'-O-gallate)

Variability in procyanidin content (**Figure 57**) was relatively similar to that observed for anthocyanins; the median value was just 9 % except for CS-25 and TE-50 (12 and 22 %, respectively). The upper and lower CV values ranged between 10 % and 22 % in CS and between 5 % and 17 % in TE, with dimers showing a higher CV, which indicated that reproducibility was in general terms very satisfactory, particularly in TE, where it was almost always below 10 %. In both CS and TE, the lower CVs were associated with 10 L and 100 L volumes. In general, the effect of tank size on procyanidin content repeatability was less relevant than it was for anthocyanins.

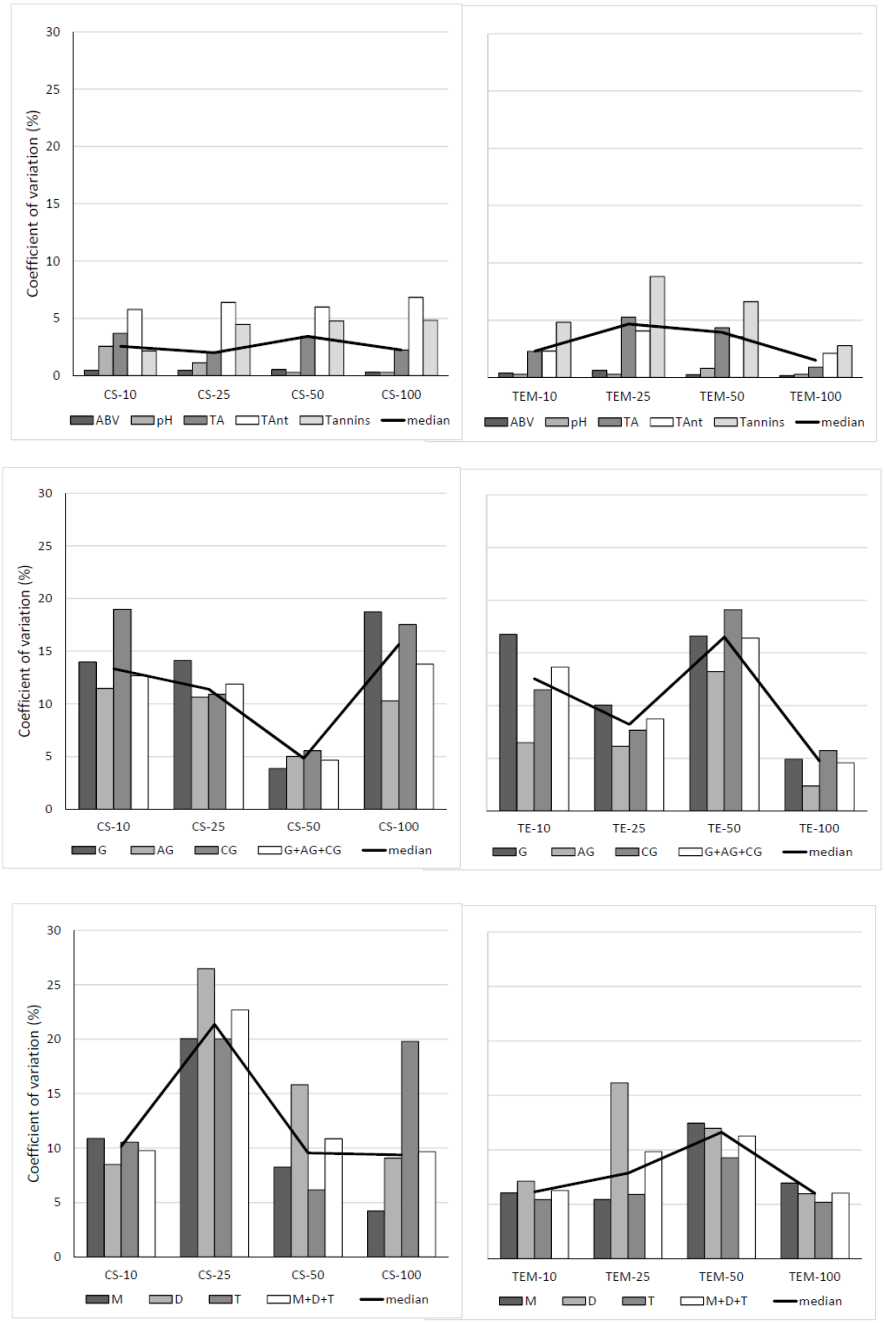

***Figure 57**. Coefficient of variations (%) for Cabernet Sauvignon (CS) and Tempranillo (TE). ABV: Alcohol by volume. pH, . TA: titratable acidity. T Ant: Total anthocyanins. G: glucosides. AG: acetyl glucosides. CG: coumaroyl-glucosides. M: monomers. D: dimers. T: trimers.*

5.4.4 Comparison with commercial volume

PCA allowed the information provided by all the analysis variables included in the study to be condensed into a reduced number of components, with a minimum loss of information in both varieties (**Figure 58**). Thus, in CS, the first component accounted for 44.6 % of variability, the second for 36.6 %, and the third for 5.9 % (**Figure 59**); whereas in TE the corresponding values were 44.0 %, 27.3 %, and 7.2 %, respectively (**Figure 60**). In both varieties, the first component included mainly anthocyanin-content variables, the second

included procyanidins-content variables, and the third component was linked predominantly to acidity (pH in CS, and TA in TE).

PCA scores for all small-scale tanks, average scores for each small-scale volume, and commercial scale tank scores are shown in **Figure 59** and **Figure 60** for CS and TE, respectively. For both varieties, the composition of the wine obtained in the 100 L tanks was clearly more similar to the commercial-scale wine for the main (first) component, related to anthocyanin content. For the second component, related to procyanidins, wines obtained in 10 L and 100 L volumes were the most similar to the commercial scale in CS, whereas for TE, differences were smaller in this axis, with 10 L, 25 L, and 100 L showing similar scores for this component compared with the commercial scale wine (**Figure 59** and **Figure 60**).

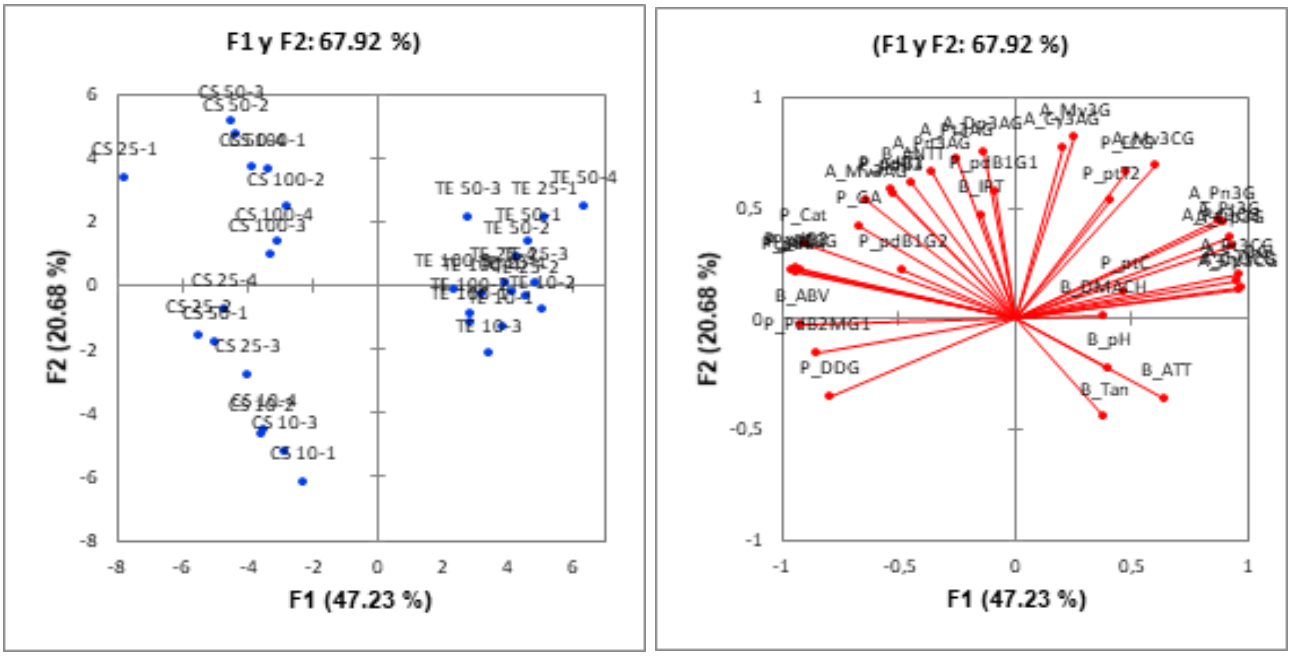

Figure 58. *Contribution of wine composition variables to Principal Component Analysis dimensions 1 and 2 in all the small-scale fermentations***.** *The resulting components from this transformation shows that the first two principal component have the highest variance and accounts for as most of the variability in the data. The first 2 components contribute to 67.92% of the total variance. Choosing two components is good enough to show that the two grape and small-scale fermentations are well separated. This justifies that we do a separate analysis of the main components of each variety.*

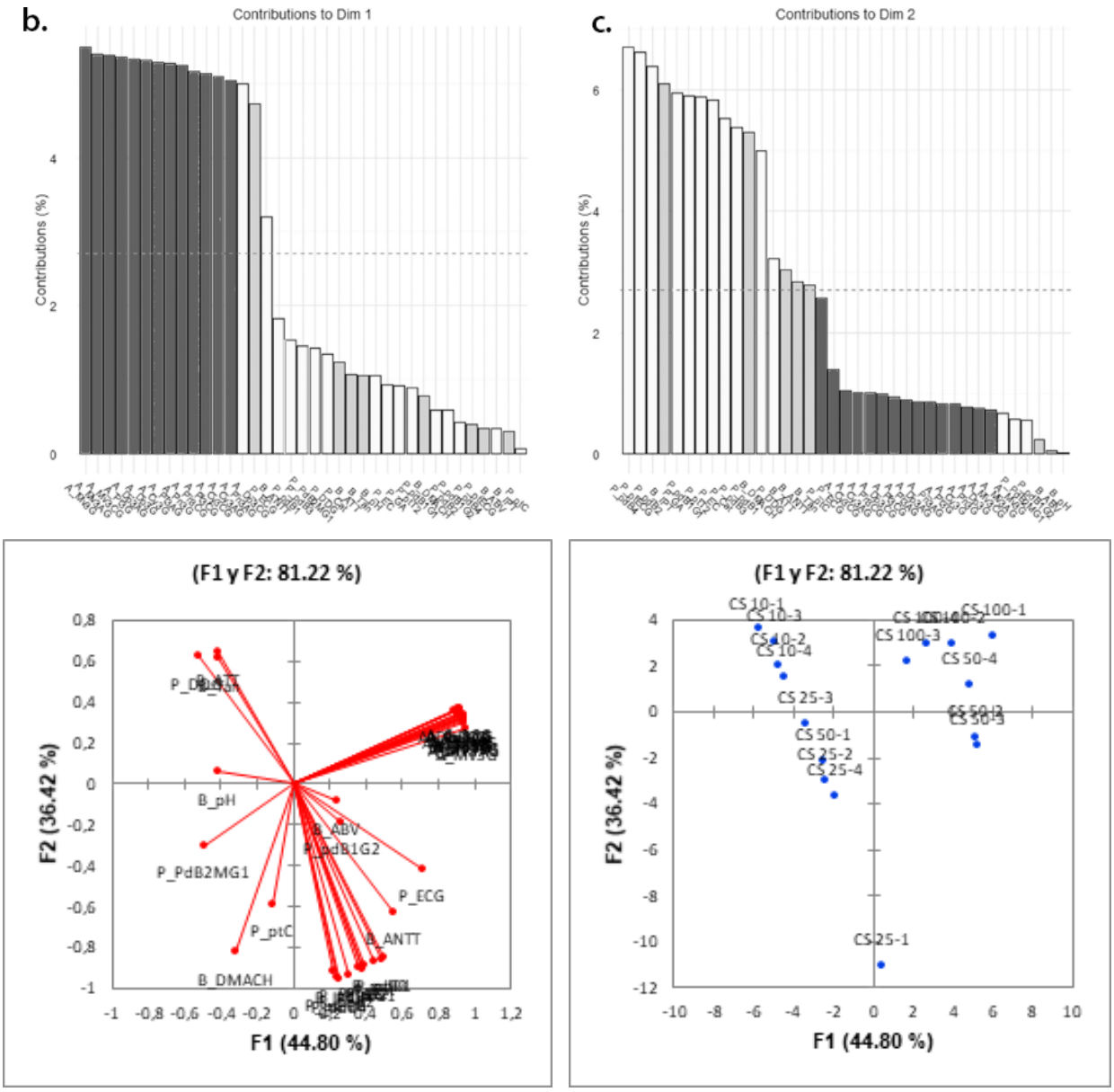

Figure 59. *Contribution of wine composition variables to Principal Component Analysis dimensions 1 and 2 in Cabernet Sauvignon. The first 2 components contribute to 81.22% of the total variance. The commercial vessel (2500 L) was considered as a supplementary individual, i.e. not including it to calculate the principal components (PC) but evaluating its performance. Those variables contributing the most are drawn darker (a,d), and have been grouped according to their family in b, c, e and f as clear grey (basic wine parameters), dark grey (anthocyanins) and white (procyanidins).*

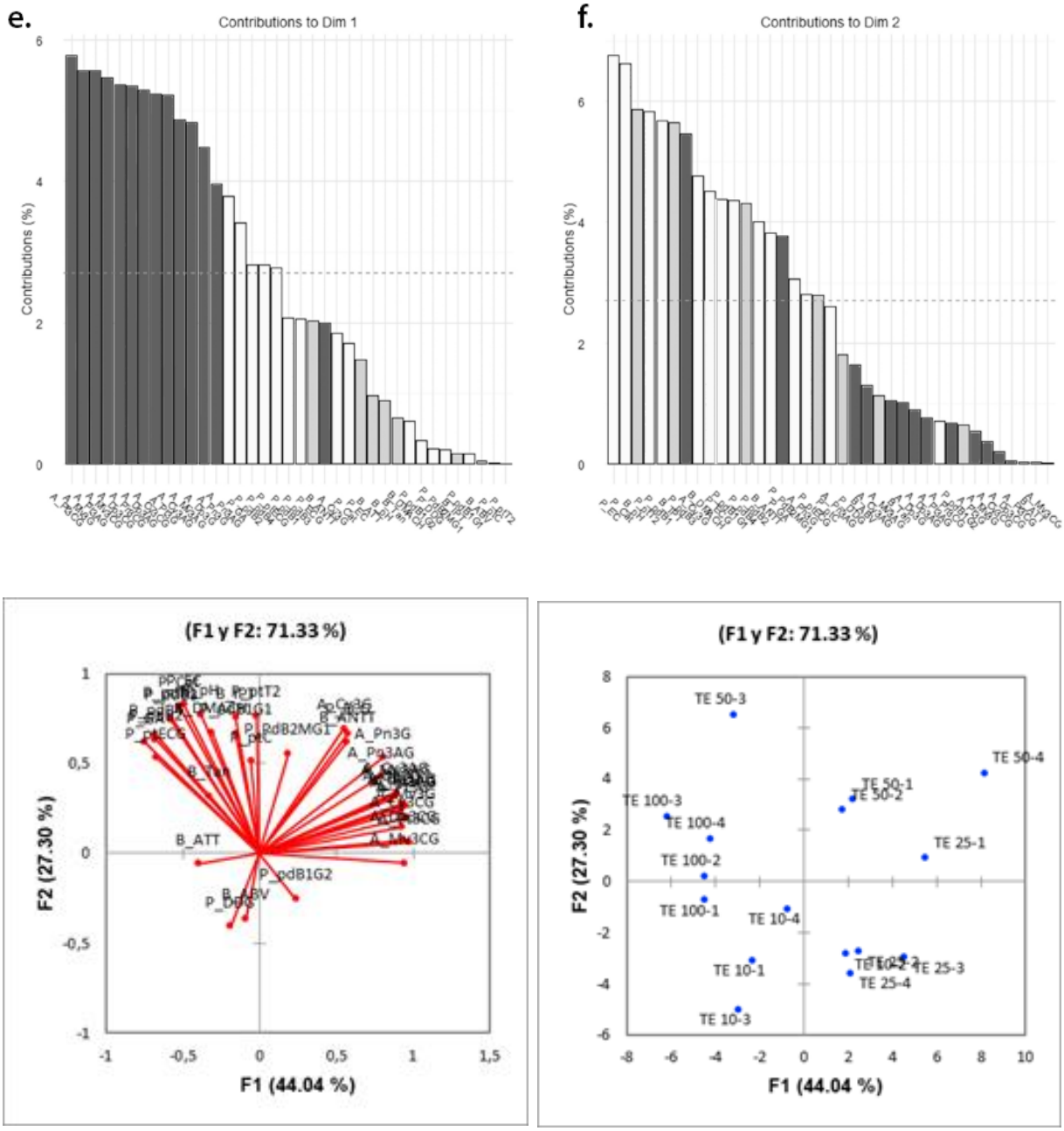

Figure 60. *Contribution of wine composition variables to Principal Component Analysis dimensions 1 and 2 in Tempranillo. The first 2 components contribute to 71.33% of the total variance. The commercial vessel (2500 L) was considered as a supplementary individual, i.e. not including it to calculate the principal components (PC) but evaluating its performance. Those variables contributing the most are drawn darker (a,d), and have been grouped according to their family in b, c, e and f as clear grey (basic wine parameters), dark grey (anthocyanins) and white (procyanidins). In both cases ABV and TTA do not contribute to distinguish the small volume vessels. Contrarily, Anthocyanin's contribution is needed to explain variables in Dimension 1.*

5.5 Conclusions

Although other studies investigating microscale fermentation have shown results using much smaller volumes (1 L), our contribution focuses on the relevance of volume fermentation size, even when larger volumes are considered. With regards to repeatability, all tank sizes proved to be adequate, since CV values were low in general. A certain trend of increased variability in 25 and 50 L tanks was observed, but the differences were small, and the CVs obtained were very satisfactory (usually below 15 % when determining phenolics and 5 % on grape and wine composition). This is an important result, since one of the main concerns of researchers in viticulture and enology is that reducing tank size in their experiments can increase variability during the fermentation stage, thus producing less reliable results. According to our data, decreasing the tank size from 100 L to 10 L does not cause an increase in variability and, therefore, the reliability of the results is very good.

However, having similar reliability in terms of variability does not mean that tank size did not affect wine typicality. For both varieties, we observed that the greatest volume was more representative of commercial scale fermentation, particularly for anthocyanins (first component in PCA). Thus, 10 L tanks achieved the lowest concentration of anthocyanin and phenol extraction into the wine, with the benefit of extraction of non-acylated anthocyanins. De Villiers *et al*. (2004) found that non-acylated glycosides are more easily extracted, followed by acetyl glycosides, and p-coumaroyl; the latter being more difficult to extract from grapes to wine. Alternately, procyanidins, included predominantly in the second component of PCA, were extracted in larger quantities in the commercial-sized tank, although 10 L, 25 L, and 100 L showed similar scores for this component compared with commercial-scale wine. The pump-overs and extended maceration that takes place in commercial wine may have a different effect when compared to the gently hand-punched action used on the small-scale. This may be due to the additional mechanical action of the pump, which does not apply to small volumes and leads to a much greater concentration of monomers moving into the wine. However, despite different extraction of monomers, dimers, and trimers, the total procyanidin content was more similar between tanks than that observed in the extraction of anthocyanins. Tank size affected fermentation dynamics in both varieties, with the effects being clearer in CS tanks, where fermentation took place more slowly due to the smaller berry size. In both varieties, the smallest tank (10 L) fermented the fastest (with no differences found between the remaining three sizes in TE) and gradually fermented more slowly as the tank size increased in CS. However, tank size did not affect the total time required to complete fermentation.

Overall, according to our results, the smallest tank size used in this study could be sufficiently representative when the goal of winemaking is to compare different fields or winemaking strategies (i.e. viticulture practices or yeast trials), as variability was not affected by tank size. Nevertheless, when the objective of small-scale winemaking is to examine wine extraction and phenolic composition, mainly for red phenolic varieties, an increase in the tank volume (up to 100 L) is needed to obtain comparable results to commercial-scale wines.

In conclusion, small-scale winemaking is a valuable tool for viticulture and enological research, although small-size tanks should only be used when the objective of the research is to compare different fields or winery treatments in relative terms. However, to approach the reality of wineries, the methodology used in this article helps to identify true applicability between small-scale and large-scale fermentations to define the phenolic extraction of different grape styles for commercial wines. Larger volumes (100 L) must be used for evaluating the phenolic composition of red grapes, as small vessels (less than 100 L) would compromise research to estimate commercial phenolic extraction levels.

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Chapter 6. Influence of geographical origin of wines on the estimated polyphenol consumption in humans

6.1 Chapter summary

Two important families of polyphenol compounds present in grapes are known to influence the final wine quality: proanthocyanins (condensed tannins) and anthocyanins. Polyphenol composition is due not only to the type of cultivar but also to the location where the grapes are grown, environmental and management practices as well as the growing season. The vines grow in two climatically and geologically different villages in the Spanish well-known grape growing area Priorat. The climate of the region allows the vines to synthesize much more of these phenols and the wines from this region have especially high polyphenolic composition, particularly procyanidins and anthocyanidins. Phenolic compounds of wine have also attracted much interest due to their antioxidant properties and their potentially beneficial effects for human health. The apparent low bioavailability of anthocyanins seems to cast doubt on their ability to exert their proposed beneficial effects throughout the body. Evaluating within the literature the effects of wine on health, there is no clear evidence of what kind of wine is supposed to have more protective effects against metabolic syndrome. Based on recent studies, meta-analysis and pooled analyses on wine composition and due to its predicted low bioavailability, we estimated the efficacy intake of 5 geographical different wines (Estate Wines), according to recent research made on gastrointestinal absorption and alcohol intake effect on metabolic syndrome, to better estimate whether geographical origin of wine might have an influence on the daily antioxidant serum composition. The evaluation of different wine/doses let us suggest that the choice of a specific Estate wine in our daily meal could lead to similar levels of polyphenols, while avoiding wines with a higher alcoholic degree.

Background

Proanthocyanins and anthocyanins, two important families of polyphenolic compounds in grapes, influence wine quality. The polyphenol composition of wine depends on the type of cultivar, location, environmental conditions and management practices. Phenolic compounds have additionally attracted considerable research interest due to their antioxidant properties and potential beneficial effects on human health. However, the low bioavailability of anthocyanins creates a major bottleneck in their ability to exert beneficial effects. Despite extensive research on the effects of wine on human health, no clear evidence has emerged on the benefits of wine quality or geographic area of production on adverse health conditions, such as metabolic syndrome.

Scope and Approach

Five climatically and geologically distinct areas from the famous Spanish grape-growing area, Priorat, were evaluated. Owing to the poor rainfall and scarcity of water during harvest, vines synthesize significant amounts of polyphenols. Based on recent studies, meta-analyses and pooled analyses of wine composition along with the predicted low bioavailability of polyphenol compounds, we estimated the efficacy of five geographically distinct wines according to gastrointestinal absorption and effects of alcohol intake in both men and women, with a view to ascertaining whether geographical origin influences the antioxidant serum composition of wine.

Key findings

Data on estimated consumption of wine suggest that the polyphenol contents are similar regardless of choice of wine/area while different alcohol compositions affect the level of alcohol and calorie intake. Thus, moderate alcohol drinkers should be advised to continue the habit, but without exceeding the dose considered a healthy threshold (up to 30–40 g of alcohol/day in men and 10–20 g of alcohol/day in women), given no medical contraindications are present.

6.2 Objectives

Although the bioavailability of wine polyphenols is known to be very low, despite of its beneficial health effects, it is not well described what category of wines –table wines, DOQ wines, etc.- might add better qualities in polyphenolic composition, considering healthy daily intake amount of food.

Polyphenols intake for each individual depends on the total amount of food containing these substances. In the case of wine, the total amount that is recommended in healthy people has some limits, due to the toxic effect of alcohol consumption. As a consequence, this report tries to evaluate how different ranges of wine recommended intake (supposed healthy) can have a beneficial effect depending on gender and age. Thus, both women and men were theoretically evaluated according to the different recommended healthy dietary intake amounts.

The aim of this study is to evaluate the influence of different Estate wine compositions on the estimated polyphenol consumption in humans. We carried out complete analyses of 5 Priorat Estate Wines to evaluate how different intake levels of wine could give the serum a specific amount of polyphenols. We also evaluated the influence of the altitude and sun-exposure on polyphenolic composition in the 5 Estate Wines. Additionally, the caloric effect of alcohol was also calculated and the decision of considering different wine doses

was to evaluate the effect of alcoholic degree of each wine, in order to see how the amount of ethanol in these wines can influence total caloric diet due to wine consumption.

6.3 Definition of Estate wine or Vinos de Pago

Wines are designated *Estate Wines, Estate Bottled* or *Vinos de Pago* if they are derived from a viticultural area with specific geological and microclimatic conditions that facilitate growth of grapes from which wines with singular qualities and traits are obtained. Specific atmospheric conditions and soil composition of a vineyard can therefore generate a range of wines that are different and unique compared to those from neighboring regions.
Vinos de Pago are a Spanish IGP (Indicación Geográfica Protegida) controlled by the *Ley de la Viña y el Vino (2003)* and the wines produced need to fulfill the updated legislation below:

1) Any single estate or Pago must be known by a name related to the place of vine growth and encompass an area less than its municipality region.
2) An estate or Pago belonging to a Denominación de Origen Calificada can be designated 'Vino de Pago Calificado'.
3) Winemaking and bottling must be conducted by the owner of the vineyard using the grapes grown in the Pago and wine must be aged and stored separately from other wines not produced from the Pago.
4) Winemaking must follow a strict quality control procedure from vine to market.

Wines used in our study belong to the Appelation of Origin Priorat, which is considered a Denominación de Origen Calificada and thus labeled 'Vinos de Pago Calificado'.

6.4 Grape polyphenolic synthesis, structure and composition

Two main pathways are implicated in the biosynthesis of phenolic compounds: shikimic acid and malonic acid (Ávalos Garcia et al., 2009). The malonic acid pathway is considered one of the most important sources of phenols in fungi and bacteria but less extensively used in superior plants. On the other hand, the shikimic acid pathway is responsible for biosynthesis of the vast majority of polyphenolic compounds in plants. Starting from erythrose-4-phosphate and phosphoenolpyruvic acid, a sequence of reactions is initiated leading to the generation of shikimic acid and a number of aromatic amino acids (phenylalanine, tryptophan and tyrosine). Most polyphenolic compounds are derived from phenylalanine. Phenolic compounds are important contributors to antioxidant properties and the colour and mouthfeel of red wine (Singleton and Rossi, 1965). Two important families of polyphenol compounds present in grapes are known to influence final wine quality, specifically, proanthocyanidins (condensed tannins) and anthocyanins. The former contribute to the astringency and bitterness of wines while the latter are pigments responsible for wine colour (Lea and Joworsky, 1987;Lea, 1992). Polyphenol composition is attributed not only to the type of cultivar but also location of grapes, environmental and

management practices, and the growing season (Romeyer et al., 1983) (Singleton and Trousdale, 1983;Jackson and Lombard, 1993) . Proanthocyanidins and anthocyanins constitute the two most abundant classes of phenolic compounds in berry skin. Condensed tannins are polymeric flavan-3-ols mainly comprising subunits of (-)-epicatechin in addition to significant amounts of epigallocatechin, (+)-catechin, and epicatechin- 3-*O*-gallate (Harborne and Grayer, 1988).

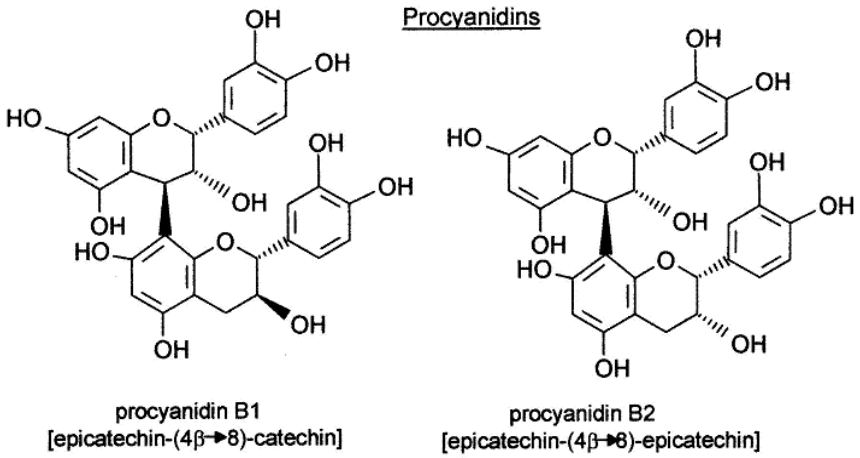

Figure 61. *Procyanidin structure. The different subunits are linked by C4-C8 and, to a lesser extent, C4-C6 interflavan bonds.*

Anthocyanins are responsible for the colour of red and black varieties of grapes. Most *Vitis Vinifera* varieties produce non-acylated glucoside, acetyl glucoside, coumaroyl glucoside (and, to a lesser extent, caffeoyl glucoside) derivatives of delphinidin, cyanidin, petunidin, peonidin, and malvidin. Each variety of grape has a specific anthocyanin profile. Anthocyanin analysis has been proposed for varietal authentication of grapes and wines. Both anthocyanins and tannins are partially extracted from grape skin during wine making and undergo structural transformations through several reactions with significant influence on wine sensory characteristics due to their involvement in astringency, bitterness, colour intensity, and colour stability (Brouillard, 1988).

Anthocyanins represent the largest group of water-soluble pigments in the plant kingdom. These compounds are widely distributed in crops, beans, fruits, vegetables and red wine, resulting in human ingestion of significant amounts of anthocyanins from plant-based daily diets. In general, anthocyanin pigments are stable under acidic conditions but are unstable and rapidly broken down under neutral conditions.

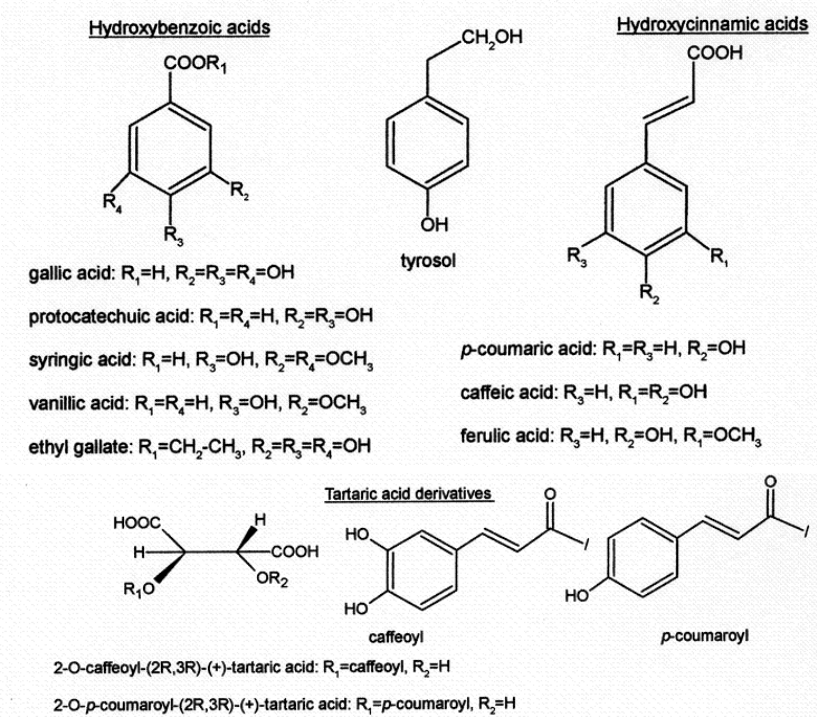

***Figure 62**. The main phenolic acids are also found in wine: hydroxybenzoic acid, Tyrosol, Hydroxycinnamic acid, tartaric acid and derivatives*

Another large group of flavonoids are flavonols (quercetin, myricetin, kaempferol, isorhamnetin and their glycosides), which contribute to bitterness, red wine colour (Boulton, 2001), and antioxidant activity (Plumb et al., 1998). The concentration of phenolic compounds in grapes is also dependent on the grape cultivar and influenced by viticultural and environmental factors, such as maturity stage, seasonal conditions, production area and fruit yield (Mazza et al., 1999; Cheynier et al., 1998; Broussaud et al., 1999; Ojeda et al., 2002).

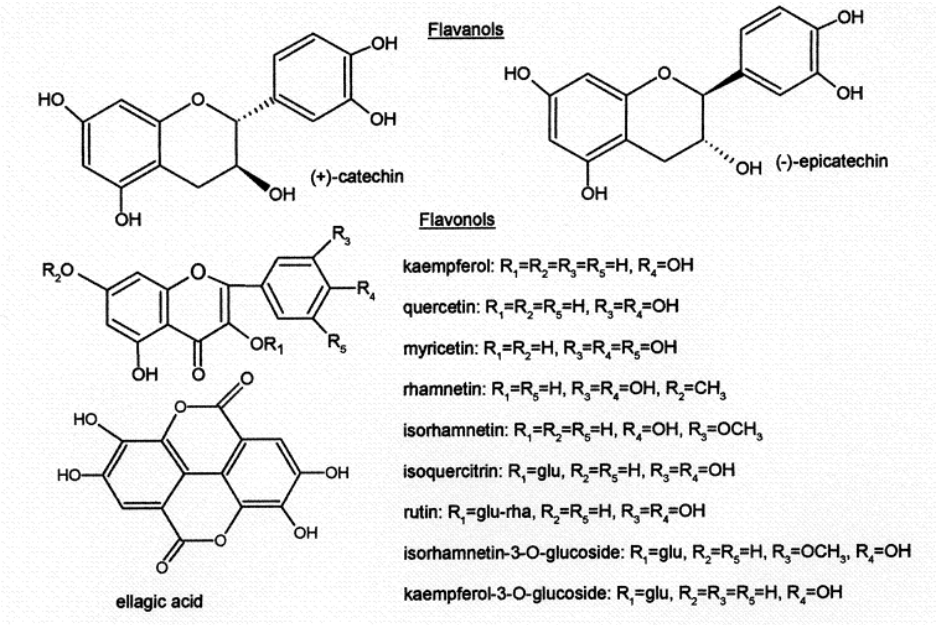

***Figure 63**. Structure of Flavanols, Flavonols and ellagic acid*

Resveratrol is synthesized in grape skin as a response to fungal infection. The compound acts as a phytoalexin, preventing pathogen proliferation. During an attack of *Botrytis cinerea* (the main fungal infection damaging wine crops), plants form a resveratrol barrier (Smidrkal, 2001). Additionally, in grape berries of some varieties, piceid, a stilbene glucoside of resveratrol is detected, which is related to the biosynthesis of resveratrol. Together with resveratrol, its oligomers (the dimer *trans*-ε-viniferin and trimer α-viniferin) have been detected in wine. Resveratrol levels in red wines range between 0.1 and 14.3 mg/L (Baur and Sinclair, 2006).

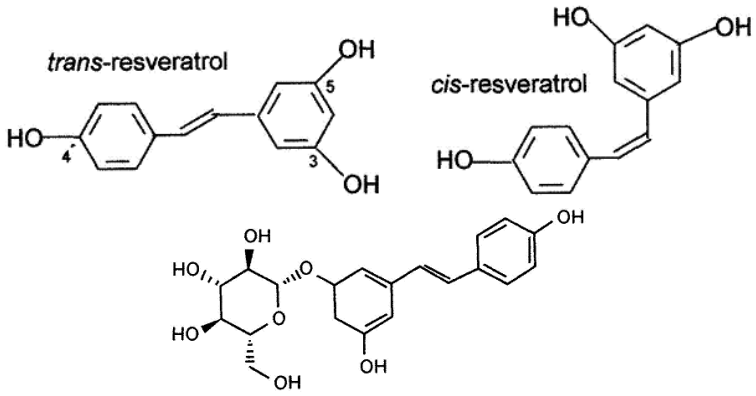

Figure 64. *Structure of piceid, resveratrol 3-O-β-D-glucopyranoside.*

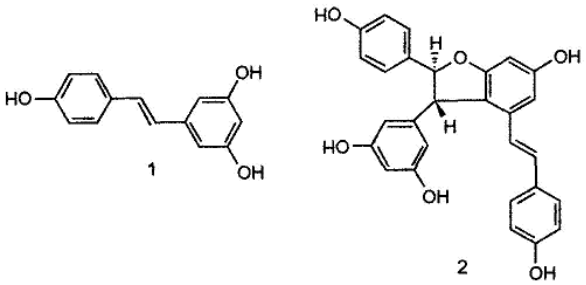

Figure 65*. Structures of trans-resveratrol (1) and its dimer* trans-*ε-viniferin (2) components of wine.*

Table 37 and **Table 38** present the main grape and wine phenolic antioxidants (including phenolic acids) and their classification.

Table 37*. Generic classification of phenolic compounds.*

Class of wine antioxidants	Compound
Flavanols	(+)-catechin (-)-epicatechin
Hydroxybenzoic acids	gallic acid protocatechuic acid syringic acid vanillic acid ethyl gallate ellagic acid
Hydroxycinnamic acids	p-coumaric acid o-coumaric acid

	caffeic acid ferulic acid
Tartaric acid and derivatives	caftaric acid (2-O-caffeoyl-(2R,3R)-(+)-tartaric acid) fertaric acid (2-O-feruloyl-(2R,3R)-(+)-tartaric acid) coutaric acid (2-O-p-coumaryl-(2R,3R)-(+)-tartaric acid)
Proanthocyanins	procyanidin B1 procyanidin B2
Phenols	Tyrosol Hydroxytyrosol 4-ethylguaiacol tryphtophol
Flavonols	kaempherol quercetin rhamnetin isorhamnetin myricetin kaempherol-3-O- glucoside isorhamnetin-3-O- glucoside isoquercitrin rutin
Anthocyanins (coumaroylated, acylated, pyranoanthocyanins)	cyanidin-3-O-glucoside delphinidin-3-O-glucoside peonidin-3-O-glucoside petunidin-3-O-glucoside malvidin-3-O-glucoside Vitisin A Vitisin B
Resveratrols	*cis*-resveratrol *trans*-resveratrol *trans*-piceid *cis*-piceid *trans*-ε-viniferin α-viniferin

***Table 38**. Phenolic compounds in different parts of grape and its products.*

Origin	**Phenolic compounds**
seed	gallic acid, (+)-catechin, epicatechin, dimeric procyanidin, proanthocyanins
skin	Proanthocyanins, ellagic acid, myricetin, quercetin, kaempferol, trans-resveratrol
leaf	myricetin, ellagic acid, kaempferol, quercetin, gallic acid
stem	rutin, quercetin 3-*O*-glucuronide, trans-resveratrol, astilbin
raisin	hydroxycinnamic acid, hydroxymethylfurfural
red wine	malvidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, catechin, quercetin, resveratrol, hydroxycinnamic acid

6.5 Effects of climate, soil and vineyard on grape composition.

Climate and wine quality are strongly linked in viticultural areas worldwide. Given the climate is sufficiently warm to ripen a specific grape cultivar, quality is inversely related to warmth and length of summer (Sánchez-Ortiz et al., 2020). A number of studies on the

climatic effect on quality suggest that wines derived from cooler climates are fresher, more acidic, and finer in bouquet and aroma while wines from warmer regions are high in alcohol content and short on taste and aroma. Vines take up only water and dissolved mineral ions from the soil and a poor soil structure will allow grapevines to send roots down. Priorat soil types are typically found on slopes and ridges with characteristic erosion and decomposition. Vines are planted on slate-driven soils and are often dry-vineyarded, receiving little or no irrigation. On the other hand, valley soils are typically more fertile and dense, composed of finer textural elements (Edo et al., 2014). Deep penetration of root systems into these soils can lead to excessive growth at the expense of concentrated flavours. Priorat DOQ, which is situated behind the coastal mountain range of Tarragona, is characterized by a Mediterranean climate (Nadal and Sánchez-Ortiz, 2011) with very little precipitation during the vegetation cycle. The soil is of poor quality, dry and pebbly, and mainly composed of slate.

6.6 Healthy effects of wine polyphenols.

Phenolic components of wine have attracted significant research interest due to their antioxidant properties and potential beneficial effects on human health (Fernández-Pachón et al., 2004; de Beer et al., 2003). Grape seed extract has been commonly used in recent years as a nutritional supplement (Waterhouse et al., 2000). However, analysis of phenolic compounds from vine and wine products (grape seeds and skins, musts, and wines) is complex due to their significant diversity. Dietary intake of polyphenols from red fruits, vegetables and red wine can be as high as 200 mg/day and their consumption via red wine has been proposed as part of the reason underlying the ''French Paradox'' (Clifford, 2000) suggesting that a diet rich in saturated fats and moderate alcohol consumption could prevent the elevated levels of heart disease, cancer and stroke found in other countries.

Anthocyanins are effective antioxidants (Stintzing and Carle, 2004) but also have other biological activities with health benefits independent of antioxidant capacity. Examples include inhibition of cancer cell growth *in vitro* (Zhang et al., 2007), induction of insulin production in isolated pancreatic cells (Jayaprakasam et al., 2005), reduction of starch digestion through inhibition of α-glucosidase activity (Matsui et al., 2001), suppression of inflammatory responses (Tall et al., 2004), protection against age-related decline in cognitive behaviour and neuronal dysfunction in the central nervous system (Joseph et al., 1999*)*. Breeding of crops with increased anthocyanin content has been an important target of research (Brennan, 1996). However, to achieve biological effects in specific tissues or organs, anthocyanins must be bioavailable, i.e., effectively absorbed from the gastrointestinal tract (GIT) into the circulation and delivered to the appropriate locations within the body. Studies on oral administration of anthocyanins have confirmed increased antioxidant status of serum (Serafini et al., 1998; Ramirez-Tortosa et al., 2001), but this is

usually accompanied by very low uptake of anthocyanins (Lapidot et al., 1998; Bub et al., 2001; Frank et al., 2003) and corresponding low levels of urinary excretion as intact or conjugated forms. The apparent low bioavailability of anthocyanins casts doubt on the ability to exert their proposed beneficial effects in the human body. Anthocyanins are therefore not generally recognized as a physiological functional food factor. However, cyanidin 3-glucoside (C3G), a typical anthocyanin, is reported to exert antioxidative and anti-inflammatory effects *in vitro* and *in vivo* (Tsuda et al., 1998) (Tsuda et al., 1999) (Tsuda et al., 2002) (Tsuda et al., 2002) (Tsuda et al., 2003), clearly suggesting beneficial effects beyond its antioxidant capacity.

Epidemiologic studies have linked flavonoid-rich foods with reduced risk of cancer and cardiovascular disease. While the mechanisms underlying the suggested health benefits of flavonoid-rich foods remain to be fully elucidated, *in vitro* and *in vivo* studies have demonstrated that flavanols and procyanidins from wine have a number of beneficial biological activities, including the ability to reduce oxidative damage, promote endothelium-dependent relaxation, and decrease platelet aggregation.

6.6.1 Metabolic syndrome

Metabolic syndrome is a combination of several clinical features including central obesity, high blood pressure, elevated fasting glucose and triacylglycerol (triglycerides) contents, along with low concentrations of HDL cholesterol, and insulin resistance. The clustering of these features is speculated to increase the risk of cardiovascular disease, which is associated with each component. Consistent with this theory, Isomaa et al., (2001) have reported that metabolic syndrome markedly increases cardiovascular morbidity and mortality. Metabolic syndrome components include: *1*) central obesity measured as waist circumference (102 cm for men and 88 cm for women), *2*) high serum triacylglycerol (150 mg/dL), *3*) low serum HDL cholesterol (40 mg/dL for men and 50 mg/dL for women), *4*) hypertension (systolic/diastolic pressure greater than 130/85 mmHg, and *5*) high fasting glucose (greater than 110 mg/dL). Metabolic syndrome is defined as the presence of three or more of these components.

6.6.2 Alcohol intake

Limited studies to date have focused on the effects of alcohol on development of metabolic syndrome. While an association between alcohol drinking and prevalent metabolic syndrome has been documented, the findings are inconsistent. Some studies indicate that the relationship is inversely linear (Park et al., 1998; Djousse et al., 2004) , J-shaped (Yoon et al., 2004) or positively linear (Fan et al., 2006) whereas others show no association (Lee at al., 2005). In addition, the association appears to differ based on type of alcoholic beverage. Compared with no alcohol consumption (Motilva et al., 2016), light

to moderate drinking of wine and beer appears favourable for reducing the prevalence odds ratio of metabolic syndrome whereas liquor drinking tends to increase the ratio or have no association with metabolic syndrome. Earlier studies on the association between alcohol consumption and metabolic syndrome have had limited success in establishing causality owing to their cross-sectional design (Sun et al., 2019) . To evaluate the effect of alcohol on development of metabolic syndrome, the incidence of metabolic syndrome was prospectively examined in relation to alcohol consumption status, including average daily amount consumed, type of alcoholic beverage most consumed, and drinking frequency (Inkung and Shin, 2008) and concluded that heavy drinking, in particular among liquor drinkers, is associated with an increased risk of the metabolic syndrome by influencing its components. But did not clarify the association between drinking minimal alcohol and the metabolic syndrome as well as the beverage specific association for drinking beer or wine. Additionally, a prospective study on a Korean cohort aged 40–69 years showed that heavy drinking, particularly liquor, is associated with increased risk of metabolic syndrome through affecting its components, including waist circumference, triacylglycerol content, blood pressure, and glucose. Although mounting evidence strongly supports beneficial cardiovascular effects of moderate red wine consumption (one to two drinks per day; 10-30 g alcohol) in most populations, clinical advice to abstainers to initiate daily alcohol consumption has not yet been substantiated in the literature and must be considered with caution on an individual basis (Lippi et al, 2010). Further research is warranted to clarify the association between the level of alcohol consumption and metabolic syndrome risk as well as the beverage-specific association in terms of beer or wine.

6.6.3 Coronary heart disease (CHD)

Coronary heart disease (CHD), also known as coronary artery disease, is narrowing of the small blood vessels that supply blood and oxygen to the heart. CHD is usually caused by atherosclerosis, which occurs when plaque builds up in the walls of arteries, leading to narrowing. With the narrowing of coronary arteries, blood flow to the heart can slow down or stop, reducing the delivery of oxygen to the heart and causing chest pain (stable angina), shortness of breath, heart attack and other symptoms. Cardiovascular disease is the main cause of mortality in industrialized countries but incidence rates show marked geographical differences. The lower incidence of CHD in Mediterranean countries has been partly ascribed to dietary habits. Recent findings from studies in a large European cohort suggest that a high degree of adherence to the Mediterranean diet is associated with reduction in mortality. In small-scale clinical studies, the Mediterranean diet or some of its components have been linked to reduced blood pressure along with improved lipid profiles (Hertog et al., 1995) and endothelial function. High blood pressure (HBP) is a serious condition that can trigger CHD and other health problems. Blood pressure refers to the force of blood pushing against the walls of arteries as the heart pumps out blood.

Consistently increased blood pressure over time can damage the body in many ways (Puddley and Beilin, 2006). Alcohol intake from any type of alcoholic beverage appears beneficial, but some studies suggest that red wine confers additional health benefits. The benefits of red wine are further supported by a meta-analysis of 13 studies involving 209,418 participants that showed a 32% risk reduction of atherosclerotic disease with red wine intake, which was greater than 22% risk reduction upon beer consumption (Liberale et al., 2019). Dietary intake of flavanones, anthocyanidins and specific foods rich in flavanoids is potentially associated with reduced risk of death due to cardiovascular heart disease.

Conversely, other investigations have failed to demonstrate beneficial effects of red wine, leading to the conclusion that additional lifestyle factors, such as diet, exercise, socioeconomic status or pattern of alcohol consumption, potentially play a role in the lower rates of atherosclerosis in wine drinkers (Opie and Lecour, 2007). However, increased alcohol consumption for the purpose of cardio protection cannot be justified. There is no rational reason for non-drinkers to start consuming wine as a preventive measure, considering that several other well-proven therapies exist for cardiovascular risk reduction, such as exercise, smoking cessation, blood pressure control and lowering of cholesterol (Szmitko and Verma, 2005).

6.6.4 Dyslipidaemia and diabetes

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins (chylomicrons, VLDL, IDL, LDL and HDL) that facilitate transport of lipids, such as cholesterol and triglycerides, within the water-based bloodstream. In healthy individuals, ~30% blood cholesterol is carried by HDL. HDL is proposed to remove cholesterol from atheroma within arteries for transport to the liver for excretion or re-utilization. Therefore, HDL-bound cholesterol is sometimes known as "good cholesterol" or HDL-C. A high level of HDL-C could protect against cardiovascular diseases and, conversely, low HDL cholesterol levels (<40 mg/dL or ~1 mmol/L) increases the risk of heart disease. Cholesterol contained in HDL particles is considered beneficial for cardiovascular health, in contrast to "bad" LDL cholesterol.

Recent attention has focused on food factors that may be beneficial in preventing body fat accumulation and reducing the risk of diabetes and heart disease. Although a number of drugs that target obesity-related metabolic diseases or prevent body fat accumulation have been marketed, little evidence showing that food factors are directly beneficial in improvement of dysfunction of adipocytes responsible for adipocytokine secretion and lipid metabolism is available (Ardevol et al., 2000). Anthocyanins were recently shown to enhance adipocytokine (adiponectin and leptin) secretion, expression of PPARγ and adipocyte-specific genes in isolated rat adipocytes without stimulation by PPARγ ligand

activity for the first time (Tsuda et al., 2004). However, other anthocyanin-responsive genes may exist that would contribute to clarification of the biological basis for utilization of anthocyanins as physiological functional food factors. Nutrigenomics is the application of high-throughput genomic tools in nutrition research. Significant advances in DNA microarray technology should promote our understanding of anthocyanin-mediated influence on gene expression and regulatory mechanisms of genes responsible for prevention of obesity and amelioration of insulin sensitivity through modulation of adipocyte function. Data from DNA microarray analysis showed for the first time that anthocyanins enhance the lipolytic activity and gene expression of related enzymes in adipocytes (Tsuda et al., 2005). Dietary anthocyanin has recently been shown to significantly suppress the development of obesity. A number of studies suggest that anthocyanins regulate obesity and insulin sensitivity associated with adiponectin and leptin secretion and PPARγ activation in adipocytes.

The normal non-diabetic blood glucose level ranges from 70 to 110 mg/dl, depending on the type of blood tested. Glucose level >140 mg/dl is usually indicative of diabetes (except in newborns and some pregnant women). Insulin, a hormone made by the pancreas, helps the body utilize glucose for energy. Insulin resistance is a condition in which the body produces insulin but cannot use it properly. In individuals with insulin resistance, the muscle, fat, and liver cells do not respond normally and require more insulin for glucose entry into cells. Eventually, the pancreas fails to keep up with the body's surplus need for insulin. Excess glucose builds up in the bloodstream, setting the stage for diabetes. Patients with insulin resistance often have high levels of both glucose and insulin circulating in the blood.

Insulin resistance (Yamauchi et al., 2001) increases the risk of developing type 2 diabetes and heart disease. Atherosclerotic diseases are prevalent as secondary complications associated with type 2 diabetes, and a diet high in readily absorbable carbohydrates is associated with increased risk of type 2 diabetes (Shulze et al., 2004). Accumulating epidemiologic data implicate postprandial hyperglycaemia as a risk factor in the development of cardiovascular disease. Elevated postprandial glucose levels may have a direct toxic effect on the vascular endothelium mediated via oxidative stress, independent of other cardiovascular risk factors, such as hyperlipidaemia (Griendling and FitzGerald, 2003). Postprandial hyperglycaemia also may exert effects through its substantial contribution to total glycaemic exposure. Ischemia-reperfusion causes oxidative damage that is enhanced with repetitive postprandial hyperglycaemia (Franzt et al, 2005). Among the cells damaged by diabetes are primary sensory neurons, also known as dorsal root ganglion neurons. Damage to these cells triggers diabetic peripheral neuropathy. Elevated glucose leads to apoptosis in neurons (Vincent et al., 2005) accompanied by increased oxidative stress. Procyanidins have insulin-like effects in insulin-sensitive cells that could explain their antihyperglycemic effect *in vivo*. These effects, in addition to their antioxidant activity, may contribute to beneficial effects against diabetes (Pinent et al., 2004). Earlier

epidemiologic studies indicate that alcohol consumption is associated with improved insulin sensitivity but experimental evidence to confirm this finding is limited. For instance, moderate wine consumption by overweight women in a previous study did not improve or impair insulin sensitivity or induce changes in any of the known indicators of insulin sensitivity, including body weight and composition, blood lipid, and blood pressure (Cordain et al., 2000).

6.7 Bioavailability of anthocyanins and procyanidins

Bioavailability refers to the amount of a specific nutrient in food or a bioactive ingredients ultimately used by the body to perform specific physiological functions, becoming available at the site of action after absorption from the gastrointestinal tract (McGhie and Walton, 2007). Several factors influence nutrient bioavailability, including digestion, absorption, distribution in blood and entry into tissue where it is physiologically effective. Bioavailability can be quantified to some extent by measuring: (1) the amount of the nutrient in various body tissues and fluids or (2) growth or enzyme activity dependent on the nutrient. However, a nutrient is rarely stored in a single body tissue, and therefore, determining the levels in single tissues does not accurately reflect true bioavailability (Cavalgante et al., 2018). For example, levels of nutrients in blood, which is easily accessible for measurement purposes, may not reflect those in other tissues that serve as major stores, such as liver. Each step involved in the process that facilitates bioavailability of nutrients is affected by a variety of factors in food and the nutritional status of individuals. It is particularly difficult to assess bioavailability in cases where the nutrients are present in many different forms in foods and tissues.

While the flavanol monomers in wine ((-)-epicatechin and (+) catechin) are readily absorbed and metabolized in humans (Williamson and Manach, 2005), little is known about the bioavailability and metabolism of procyanidins. Several studies have shown rapid absorption of polyphenolic compounds, such as procyanidins, quercetin and flavanols, from grapes into plasma (Bell et al., 2000) (García-Alonso et al., 2006) (Baba et al., 2001). After two weeks of daily red wine consumption (375 mL), total plasma phenol concentrations increased significantly and trace levels of metabolites from (+)-catechin and (-)-epicatechin were detected in plasma. However, the biotransformation and bioavailability patterns of many dietary polyphenols remain to be clarified, particularly anthocyanins (Fernandes et al., 2015) The tissue distribution and biotransformation pathways for several dietary polyphenols are yet to be determined. Furthermore, biological activities of metabolites of many dietary polyphenols require further investigation. The potential health benefits of dietary polyphenols require confirmation in both animal models of disease and humans at appropriate doses. Whereas *in vitro* studies have provided insights into the mechanisms of action of individual dietary polyphenols (Han et al., 2020), the significance of these findings requires validation *in vivo*. Further efforts should be made to integrate the available *in vitro* and *in vivo* activity data with

bioavailability data for assessing the potential utility of various dietary polyphenols. Accumulating evidence from human feeding studies suggests that the absorption and bioavailability of specific flavonoids are markedly higher than originally believed (Ross and Kasum, 2002). Most flavonoids in plants are attached to sugars (glycosides) and occasionally exist as aglycones. Aglycones are freely absorbed from the gut by passive diffusion while glycosides are hydrolysed by colon microflora before gastrointestinal absorption (Zubick and Meydani, 2003).

Human feeding trials with wine have demonstrated that procyanidins can survive the acidic milieu of the stomach and are therefore not initially broken down, entering the small intestine intact. Consistent with this finding, dimer B2 [epicatechin (4β-8)-epicatechin] was detected in human plasma as early as 30 min after consumption of a flavanol-rich food. Thus, while the metabolic fate of dimer B2 is yet to be elucidated, clearly it can be absorbed, supporting a contributory role to the benefits of flavanol/procyanidin-rich food (Zhang et al., 2006). In terms of absorption, lactase phloridzin hydrolase (LPH) hydrolyzes the majority of anthocyanidins, allowing absorption by the small intestine (Crespy et al., 2001). Notably, cyanidine-3-glucoside is not hydrolysed in small intestine (Vitaglione et al., 2007). Other recent studies indicate that bilitranslocase plays a role in absorption at the gastric level (Nicolin et al., 2005). The degradation of anthocyanins mainly takes place in the intestine, where both the intestinal microbiota and pH play important roles in catabolizing anthocyanins into metabolites. The degradation products of anthocyanins in the gastrointestinal tract are reported to be phenolic acids, phenol aldehydes and phenols. Both anthocyanins and degraded products or metabolites can be absorbed through either passive diffusion or active transport. The molecular absorption mechanism is still to be fully clarified in order to assess the real in vivo digestion, absorption, bioavailability and bioactivities of anthocyanins (Han et al., 2019).

6.8 Synergy of wine polyphenols with food

Assessment of nutrient bioavailability remains critical to our understanding of the mechanisms by which humans utilize essential nutrients from consumed foods and how foods satisfy nutritional requirements (Schönfeldt et al., 2016). Different food components could reduce or enhance nutrient bioavailability. Some components form complexes with a nutrient and prevent its digestion or absorption or even induce degradation. In the case of wine, phenolic compounds are able to chelate iron and red wine decreases the concentration of digested phenolic products attributable to the formation of iron-polyphenol chelates. In terms of protein affinity, flavonoids are strongly affected by the presence of milk, especially after the digestion process (Cilla et al., 2009). Additionally, using an in *vitro* digestion procedure, Argyri et al. (2005) found that co-digestion of red wine with vitamin C and meat resulted in an increase and decrease in antioxidant capacity

and total phenol content, respectively. Similarly, co-digestion of raspberry extract with meat, bread and cereals decreased the recovery of total phenol (McDougall et al., 2005) but not anthocyanins in the serum-available fraction. The hydroalcoholic matrix of wine could facilitate the solubility and absorption of its phenolic components (Soleas, 1997).

6.9 Results and discussion

6.9.1 Sampling and winemaking

The climate in the Priorat region (Tarragona, Spain), characterized by very high temperatures during summer, drought and steep and poor stony soils, promotes ecosystem vulnerability to current global changes. A recent report on how mesoclimate influences wine quality has shown the differences between Priorat mesoclimates. The study involved five different vineyards, whereby two villages under two different mesoclimates were selected (early and late ripening and two/three different parcels in each mesoclimate, topographically located up or down the slope). At each of the two municipalities, El Molar (early) and Porrera (late), 60 years old vines were selected, planted in bush at a density of 5000-6000 vines·ha^{-1}. To evaluate the effects of topography and mesoclimate on the qualitative potential of *vitis vinifera cv Carignan* in the Priorat region, Sanchez-Ortiz et al (2020) monitored the evolution of the maturity process in the five parcels and analysed the composition of both the grapes grown and wines obtained.

Grapes were fermented after three days of cool maceration for colour extraction, followed by fermentation of all reducing sugars, addition of 20 g/hl sulphur dioxide to preserve oxidation, and finally bottling. The wine did not undergo malolactic fermentation and was therefore young, without oaking or ageing. OIV methods (International Organisation of Vine and Wine) were used to analyse alcohol by volume (ABV), total tartaric acidity (ATT), pH, total anthocyanins (Ribéreau-Gayon et al., 2003), DMACA (flavan-3-ol by derivatization with *p*-dimethylaminocinnamaldehyde) (Vivas et al., 1993), and total tannins in wine. ANOVA was performed using the general linear model procedure. The Tukey test was used for post hoc analysis (XLSTAT statistical package, EXCEL) between plots.

Alcohol by volume (ABV), pH, TTA, T Ant, and tannins were analysed. Anthocyanin content was determined following the methodology detailed in Valls *et al.* (2009) through high-performance liquid chromatography (HPLC) using a Hewlett Packard Liquid Chromatograph (Waters Corporation, Mildford, MA, USA) equipped with a Zorbax Eclipse Plus C18 Column (150 × 2.1 mm; 3.5 µm) and a Zorbax Eclipse Plus-C18 Precolumn (12.5 × 4.6 mm; 5 µm). Injection volume was 5 µL; elution was performed with a mobile phase A of HPLC-grade water (0.2 % trifluoroacetic acid) and a mobile phase B using methanol (0.2 % trifluoroacetic acid). The column temperature was set at 50 ºC and the HPLC was coupled to a Diode Array Detector (DAD). Quantifications were performed using the DAD detector, and identifications were made considering the time of flight

(TOF). A mass spectrometry (MS) detector was used to assist in the identification. Free anthocyanin content was determined using a calibration curve (based on peak area, $y = 0.7968x + 7.5756$; $R^2 = 0.9774$), which was established using malvidin 3-glucoside standard solutions submitted to the same procedure. Anthocyanidin-3-monoglucosides and respective acetylated and coumaroylated glycosides were identified on the basis of their ultraviolet–visible (UV–vis) spectra and retention times (**Table 28**). Anthocyanidins were identified by HPLC, making a comparison with internal standards. Calibration curves were obtained by injecting standards with different concentrations of malvidin 3-glucoside (Extrasynthese, Genay, France). The range of the linear calibration curves was 0.1 to 1.0 mg/L for the lower ($R^2 > 0.996$), 0.1–5.0 mg/L for intermediate ($R^2 > 0.987$), and 10.0–200.0 mg/L for the higher concentration compounds ($R^2 > 0.987$). Unknown concentrations were determined from the regression equations, and the results were expressed as milligrams of malvidin 3-glucoside. Repeatability of HPLC analysis gave a coefficient of variation of <7 %.

Procyanidins were analysed by injecting 3 µl of wine samples through Rapid Resolution Liquid Chromatography (RRLC) using a Zorbax Eclipse XDB-C18 (50 × 30; 1.8 µm) followed by a RRLC in-line pre-column (4.6 mm, 0.2 µm) at 30 ºC. The HPLC injection volume was 1.4 µL, with a 0.7 mL/min flux; mobile phase A: water (0.1 % formic acid), mobile phase B: methanol (0.1 % formic acid). Phenolic compounds were identified according to their order of elution, retention times of pure compounds (gallic acid, catechin, procyanidin dimer B2, mono gallate dimer, procyanidin trimer C1, and epicatechin gallate) and their molecular masses. **Table 29** shows the retention time and *m/z* for each compound.

6.9.2 Composition of Priorat Estate wines used for the study

In order to assess the estimated consumption of 5 Estate wines, previous analysis of individual wines was conducted. These wine were not blended with other grapes nor with vintages, thus the 5 five wines are considered single varietals and single vintage. This adds a lot of value to this study as most of the wines in the market are difficult to prove that are made from a single blend (the European law allows to label as single grape and single varietal even if this contains a minimum of 15% of other grapes and other vintages in the same blend). In this study, total anthocyanin and tannin contents, DMACH, pH, total acidity and alcohol % of each wine sample are presented in **Table 39**.

Table 39. *Total anthocyanins, tannins, DMACH, pH, total acidity and alcohol % of each wine sample.*

Estate Wine	Site 1		Site 2		Site 3		Site 4		Site 5	
Total Anthocyanins (mg/L)	398,4 ± 9,6	**a**	466,6 ± 4,0	**b**	470,5 ± 22,0	**b**	432,9 ± 22,8	**ab**	451,5 ± 6,3	**b**
Tannins (g/L)	2,9 ± 0,2	**c**	2,4 ± 0,2	**a**	2,2 ± 0,3	**a**	1,7 ± 0,1	**b**	1,4 ± 0,1	**b**
DMACH (mg/L)	388,8 ± 22,9	**a**	361,4 ± 14,4	**a**	324,0 ± 7,9	**b**	295,6 ± 8,0	**bc**	263,1 ± 4,5	**c**
pH	3,17 ± 0,1	**ac**	3,27 ± 0,0	**abc**	3,28 ± 0,0	**abc**	3,35 ± 0,0	**b**	3,21 ± 0,0	**c**
ATT (g/L)	6,63 ± 0,2	**ac**	6,40 ± 0,2	**c**	6,39 ± 0,0	**c**	6,85 ± 0,1	**a**	7,62 ± 0,1	**b**
% alc. vol	16,10 ± 0,4	**a**	15,20 ± 0,6	**abc**	14,80 ± 0,1	**bde**	14,50 ± 0,2	**ce**	15,40 ± 0,2	**ad**

6.9.3 Concentration in anthocyanins and procyanidins in the wines considered for the study

According to the previous analysis of 5 wines from 5 different geographically areas in the Priorat described by Sánchez-Ortiz (2020), wines from Site 1 and Site 2 region represent Estate wines derived from the warmest area (early ripening, E) while Estate wines from Site 3, Site 4 and Site 5 are obtained from the coldest area (late ripening, L). Estate wines are designated Site 1 (El Molar, early region, uphill), Site 2 (El Molar, early region, downhill), Site 3 (Porrera, late region, downhill west-exposed), Site 4 (Porrera, late region, downhill east-exposed) and Site 5 (Porrera, late region, uphill). Total anthocyanin contents of two Estate wines (Site 1 and Site 4) were not significantly different while estate wines from Site 2, Site 3 and Site 5 locations displayed higher anthocyanin concentrations compared to Site 1, which had the lowest content. Estate wine from Site 1 had the highest concentration of tannin, which was significantly different from tannin contents of wines from Site 2, Site 3, Site 4 and Site 5. Thus, the Estate wine produced from Site 1 contained the highest tannin and lowest anthocyanin amounts. The pH for Site 1 appeared lower than that for Site 2 and Site 3, but not to a significantly different extent. In contrast, pH differences relative to Site 4 and Site 5 were marked. Highest differences in total acidity were observed between Site 1 and Site 5 estate wines that belonged to different mesoclimatic areas. The alcohol content was markedly higher in Site 1 compared to Site 3 and Site 4 (an increase in 1.56% alc. vol in Site 1 vs. Site 4 and 1.26% alc. vol in Site 1 vs. Site 3).

Results from the chemical analyses were significantly different between the two early ripening geographical areas (Site 1 and 2) with a *p*-value <0.05. Considering the influence of only early and late regions instead of vineyard location, no marked differences in total anthocyanins, pH and total acidity were observed. In contrast, total tannin content, DMACH

and alcoholic degree (% alc by vol.) were significantly different. While no major differences were recorded in total anthocyanin contents between treatments in the first analysis of wine (**Table 39**).

Results from the HPLC analysis was used for evaluation of specific anthocyanins and procyanidins, with a view to determining the polyphenol types that are more abundant in different wines with potential health benefits. **Table 40** and **Table 41** show the previous results obtained from the 5 different wine growing areas in the DOQ Priorat.

***Table 40**. HPLC analysis of anthocyanin composition.*

Estate Wine	Site 1				Site 2				Site 3				Site 4				Site 5			
malvidin-3-glucoside	164,1	±	4,8	**a**	217,1	±	4,5	**d**	222,6	±	6,3	**cd**	203,6	±	7,4	**b**	226,8	±	1,8	**c**
petunidin-3-glucoside	7,6	±	0,4	**a**	8,8	±	2,3	**b**	7,7	±	2,0	**a**	5,5	±	1,8	**a**	6,9	±	2,0	**a**
delphinidin-3-glucoside	2,0	±	1,7	**a**	2,7	±	1,0	**b**	2,2	±	1,8	**a**	2,0	±	1,3	**a**	2,6	±	0,6	**a**
peonidin-3-glucoside	4,6	±	0,2	**b**	5,2	±	0,1	**d**	5,5	±	0,1	**e**	3,5	±	0,0	**a**	4,7	±	0,0	**c**
cyanidin-3-glucoside	0,3	±	0,0	**b**	0,3	±	0,1	**ab**	0,3	±	0,0	**b**	0,2	±	0,0	**a**	0,3	±	0,0	**b**
malvidin-3-acetylglucoside	32,3	±	1,6	**a**	57,1	±	1,9	**c**	54,8	±	0,6	**b**	59,5	±	1,8	**c**	64,6	±	1,5	**d**
petunidin-3-acetylglucoside	0,8	±	0,0	**a**	1,1	±	0,0	**d**	1,0	±	0,0	**c**	0,9	±	0,0	**b**	0,9	±	0,0	**b**
delphinidin-3-acetylglucoside	0,3	±	0,0	**a**	0,4	±	0,0	**b**	0,3	±	0,0	**a**	0,3	±	0,0	**a**	0,3	±	0,0	**a**
peonidin-3-acetylglucoside	1,5	±	0,0	**a**	1,8	±	0,0	**b**	1,9	±	0,0	**c**	1,5	±	0,0	**a**	2,0	±	0,1	**d**
cyanidin-3-acetylglucoside	0,2	±	0,0		0,2	±	0,0		0,2	±	0,0		0,2	±	0,0		0,2	±	0,0	
malvidin-3-coumarylglucoside	28,7	±	2,4	**a**	38,5	±	5,6	**b**	41,5	±	1,2	**b**	34,6	±	7,9	**ab**	37,3	±	7,6	**ab**
petunidin-3-coumarylglucoside	3,9	±	0,2	**a**	5,8	±	0,0	**c**	4,4	±	0,2	**b**	3,7	±	0,2	**a**	4,2	±	0,1	**b**
delphinidin-3-coumarylglucoside	1,3	±	0,1	**b**	1,8	±	0,0	**c**	1,2	±	0,0	**b**	1,0	±	0,1	**a**	1,0	±	0,0	**a**
peonidin-3-coumarylglucoside	2,5	±	0,3		3,0	±	0,4		2,8	±	0,1		2,0	±	0,2		2,8	±	0,0	
cyanidin-3-coumarylglucoside	1,1	±	0,0	**c**	1,2	±	0,0	**d**	1,0	±	0,0	**c**	0,7	±	0,0	**a**	0,9	±	0,0	**b**
Total Anthocyanins (mg/L)	251,1	±	11,7	**a**	345,0	±	15,9	**c**	347,6	±	12,3	**c**	319,1	±	20,7	**b**	355,5	±	13,7	**c**

HPLC analysis of the five wines revealed 15 anthocyanins, mainly 3-*0*-glucosides of malvidin, petunidin, delphinidin, peonidin and cyanidin (**Table 40**). Acetylated and coumaroylated glucosides were additionally identified. ANOVA of anthocyanin data revealed high malvidin content, as expected. *Post-hoc* analyses showed that malvidin-3-glucoside (with the largest concentration amongst all the treatment groups) was significantly higher in wines from Site 2, Site 3 and Site 4.

HPLC analysis for procyanidins were also considered leading to the identification of 15 polyphenolic compounds. No differences were found in the dimer digallate levels among the five wines (**Table 41**). ANOVA revealed the highest contents of gallic acid, procyanidin B1 and catechin polyphenolic compounds. While the highest levels were detected for procyanidin B1, amounts were significantly different among all the wines examined. Estate wines from Site 1 contained the highest amount of gallic acid, followed by estate wines from Site 2-Site 4 and Site 3-Site 5. Site 1 wines are markedly different to those derived from Site 2-Site 4 and Site 3-Site 5. Differences between the latter two groups were also observed. The patterns for catechin were similar to those of procyanidin B1. Concentrations of procyanidin B2 and B4 were higher in wine samples from Site 4.

***Table 41**. HPLC analysis of five red wines leading to the identification of 15 polyphenolic compounds.*

Estate Wine	Site 1	Site 2	Site 3	Site 4	Site 5
procyanidin B3	3,5 ± 0,2 **c**	2,4 ± 0,1 **b**	2,3 ± 0,2 **b**	2,7 ± 0,1 **b**	2,0 ± 0,1 **a**
procyanidin B1	23,7 ± 0,0 **e**	19 ± 0,2 **d**	13,7 ± 0,3 **c**	14,5 ± 0,1 **b**	12,6 ± 0,2 **a**
procyanidin B4	8,5 ± 0,2 **d**	6,3 ± 0,0 **c**	6,9 ± 0,1 **b**	9,5 ± 0,2 **a**	7,1 ± 0,2 **b**
procyanidin B2	8,6 ± 0,1 **d**	6,3 ± 0,0 **c**	6,9 ± 0,1 **b**	9,6 ± 0,1 **a**	7,1 ± 0,1 **b**
dimer monogallate	1,7 ± 0,1 **c**	2,5 ± 0,1 **a**	3,0 ± 0,1 **b**	3,1 ± 0,2 **b**	1,9 ± 0,0 **c**
procyanidin B 5.1	1,2 ± 0,1 **b**	1,2 ± 0,1 **b**	0,9 ± 0,0 **a**	0,8 ± 0,1 **a**	0,8 ± 0,0 **a**
dimer digallate	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
procyanidin B 6.6	3,0 ± 0,2 **c**	3,6 ± 0,1 **b**	4,2 ± 0,2 **a**	4,7 ± 0,1 **a**	4,2 ± 0,0 **a**
gallic acid	22,1 ± 0,3 **d**	16 ± 0,6 **c**	14,6 ± 0,5 **a**	15,3 ± 0,5 **cb**	13,8 ± 0,2 **a**
catechin	10,7 ± 0,2 **e**	7,7 ± 0,1 **d**	5,0 ± 0,1 **c**	5,4 ± 0,1 **b**	4,7 ± 0,1 **a**
epicatechin	3,2 ± 0,1 **b**	1,8 ± 0,1 **a**	2,2 ± 0,0 **a**	3,1 ± 0,0 **b**	2,6 ± 0,0 **a**
epicatechin gallate	0,1 ± 0,1 **b**	0,1 ± 0,0 **b**	0,1 ± 0,0 **b**	0,1 ± 0,0 **b**	0,0 ± 0,0 **a**
trimer C 0.6	7,4 ± 0,3 **b**	6,6 ± 0,3 **b**	5,7 ± 0,1 **a**	5,5 ± 0,5 **a**	5,0 ± 0,2 **a**
trimer C 2.4	9,8 ± 0,1 **e**	6,9 ± 0,0 **d**	4,8 ± 0,2 **c**	5,5 ± 0,1 **b**	4,2 ± 0,1 **a**
trimer C1	6,4 ± 0,2 **b**	4,1 ± 0,2 **a**	4,5 ± 0,2 **a**	6,6 ± 0,2 **b**	4,4 ± 0,3 **a**
TOTAL	109,9 ± 1,0 **c**	84 ± 0,7 **b**	74,9 ± 1,0 **a**	86,2 ± 0,9 **b**	70,3 ± 0,5 **a**

6.10 Estimation of polyphenols intake in humans

Prior to determining the influence of polyphenol intake, the effects of alcohol-derived calories from all wines were examined (see Equations in **Table 42**). Atwater factor (7 kcal/g alcohol) was used for calculation of alcohol-derived energy. Average height and weight values of individuals from the Spanish population were obtained from the Ministry of Health (Ministerio de Sanidad), estimated as 78 kg and 171 cm for men and 67 kg and 160 cm for women. Body mass index (BMI) was used to determine whether the Spanish population could be classified as normal weight, overweight, obese, or extremely obese. The values obtained indicated normal average weight of the population under study. We estimated a moderate activity level for both genders (shown in **Table 42**) representing an activity factor of 1.78 for men and 1.64 for women, as recommended by World Health Organisation (WHO). Total calorie requirements were calculated from the Harris-Benedict equation (Harris and Benedict, 1918). Calorie intake due to metabolized alcohol and % energy due to alcohol were additionally calculated, along with blood alcohol levels/day.

***Table 42**. Values and equations used for calculation of Daily Energy Needs, Alcohol Energy, % Energy due to alcohol consumption, Healthy wine (recommended volume of wine intake) and blood alcohol level (BAL). % Energy due to alcohol consumption is based on Daily Energy Needs determined using Harris-Benedict Equation.*

Table calculation equations		
Description	Unit	Calculation
Activity factor (Physical activity level, PAL)	No	Light level activity factor: 1,55 (men) and 1,56 (women) Moderate level activity factor: 1,78 (men) and 1,64 (women) Intense level activity factor: 2,10 (men) and 1,82 (women)
Daily Energy Needs, REE	kcal	Women: REE=655.1+9.56*weight+1,85*high-4,68*age Men: REE= 66.5+13,75*weight+5.0*high-6,78*age +20% light, +30% moderate and 50-75% intense activity +10% Food Induced Thermogenesis
Alcohol Energy	kcal	ml wine/day·(%alc. vol/100)*alcohol density*7Kcal/g Ethanol 7kcal/g alcohol (Atwater Factor)
% Energy due to alcohol consumption	%	%=alcohol energy/Daily Energy Needs
Healthy wine (intake of wine volume recommended)	ml/day	V (ml wine) = (g healthy alcohol/ % DA·0,789)
Blood alcohol level (BAL)	g/day	BAL = (g healthy alcohol/kg)·(60L/100kg)

Amounts of 30–40 g alcohol/day for men and 10–20 g alcohol/day for women are recommended. Thus, we considered three levels of g alcohol/day: 30, 35 and 40 for men (**Table 43**) and 10, 15 and 20 for women. (**Table 44**). Considering % alc. vol of the five different estate wines, % energy due to alcohol consumption was calculated. Values from 8.9%–11.8% total energy needs for men and 3.4%–6.7% for women were obtained. The three dose ranges allowed estimation of average alcohol-derived energy.

Procyanidin and anthocyanidin absorption levels was calculated considering the recommended healthy threshold of wine intake per day. We estimated % serum-available recovery of anthocyanin and procyanidin based on a recent review (3.9% for anthocyanins and 7.2% for procyanidins) (Mc Dougall, 2005). The final serum amount of polyphenols (either procyanidins or anthocyanidins) was evaluated considering the three levels of wine (determined earlier as 30, 35 and 40 g for men and 10, 15 and 20 g for women). A total daily dietary polyphenol intake of 200 mg/day was estimated. Final estimated plasma concentration was calculated as mg/day.

***Table 43**. Estimated calculation for men based on an average weight (78.1kg), average high (171cm), average Age (45 years old), Body Mass index22.8, Activity level (moderate), Activity factor 1.78 and Daily Energy Needs determined using Harris-Benedict Equation (2366Kcal).*

	Men														
Wine	Site 1			Site 2			Site 3			Site 4			Site 5		
Healthy alcohol serving (g/day)	30	35	40	30	35	40	30	35	40	30	35	40	30	35	40
Alcohol Energy (Kcal)	210	245	280	210	245	280	210	245	280	210	245	280	210	245	280
Healthy wine (ml/day)	233	272	311	247	288	329	253	296	338	259	302	345	244	284	325
% alc. Vol	16.1			15.2			14.8			14.5			15.4		
Blood alcohol levels/day	0.23	0.27	0.31	0.23	0.27	0.31	0.23	0.27	0.31	0.23	0.27	0.31	0.23	0.27	0.31
% Energy due to alcohol consumption	8.9	10.4	11.8	8.9	10.4	11.8	8.9	10.4	11.8	8.9	10.4	11.8	8.9	10.4	11.8

***Table 44**. Estimated calculation for women based on an average weight (67.1kg), average high (160cm), average Age (45 years old), Body Mass index 21, Activity level (moderate), Activity factor 1.64 and Daily Energy Needs determined using Harris-Benedict Equation (2087Kcal).*

	Women														
Wine	Site 1			Site 2			Site 3			Site 4			Site 5		
Healthy alcohol serving (g/day)	10	15	20	10	15	20	10	15	20	10	15	20	10	15	20
Alcohol Energy (Kcal)	70	105	140	70	105	140	70	105	140	70	105	140	70	105	140
Healthy wine (ml/day)	78	117	156	82	123	164	84	127	169	86	129	172	81	122	162
% alc. Vol	16.1			15.2			14.8			14.5			15.4		
Blood alcohol levels/day	0.09	0.13	0.18	0.09	0.13	0.18	0.09	0.13	0.18	0.09	0.13	0.18	0.09	0.13	0.18
% Energy due to alcohol consumption	3.4	5.1	6.7	3.4	5.1	6.7	3.4	5.1	6.7	3.4	5.1	6.7	3.4	5.1	6.7

***Table 45**. Estimated healthy wine dose and % gastrointestinal absorption for Men: 7.2% for phenols and 3.7% for anthocyanins. Results are presented as mg/day in three groups: < 1mg/day (low), 1<x<2 mg/day (medium) and > 2mg/day (optimum).*

		TOT. PRO mg/L	TOT. ANT. mg/L	PRO. mg/day TOT. intake	ANT. mg/day TOT. intake	% BIO. PRO.	% BIO. ANT.	PRO. diary intake %	ANT. diary intake %	PRO. EXP. serum intake mg/day	ANT. EXP serum intake mg/day	Serum PRO. EXP. Intake			Serum ANT. EXP. Intake		
												<1	1<x<2	>2	<1	1<x<2	>2
Men	Site 1	109,9	251,1	25,7	58,6	7,2	3,9	12,8	29,3	1,85	2,29		x				x
				29,9	68,3			14,9	34,1	2,15	2,66			x			x
				34,2	78,1			17,1	39,0	2,46	3,05			x			x
	Site 2	84,4	345	20,8	85,2			10,4	42,6	1,50	3,32		x				x
				24,3	99,4			12,2	49,7	1,75	3,88		x				x
				27,8	113,5			13,9	56,8	2,00	4,43			x			x
	E (Mean Site 1 and Site 2)	97,2	298,1	23,3	71,9			11,6	36,0	1,67	2,81		x				x
				27,1	83,8			13,6	41,9	1,95	3,27		x				x
				31,0	95,8			15,5	47,9	2,23	3,74			x			x
	Site 3	74,9	347,6	18,9	87,9			9,5	44,0	1,36	3,43		x				x
				22,2	102,9			11,1	51,4	1,60	4,01		x				x
				25,3	117,5			12,7	58,7	1,82	4,58		x				x
	Site 4	86,2	319,1	22,3	82,6			11,2	41,3	1,61	3,22		x				x
				26,0	96,4			13,0	48,2	1,87	3,76		x				x
				29,7	110,1			14,9	55,0	2,14	4,29			x			x
	Site 5	70,3	355,5	17,2	86,7			8,6	43,4	1,24	3,38		x				x
				20,0	101,0			10,0	50,5	1,44	3,94		x				x
				22,8	115,5			11,4	57,8	1,65	4,51		x				x
	L (Mean Site 1 and Site 2)	77,1	340,7	19,5	85,8			9,7	42,9	1,40	3,35		x				x
				22,7	100,1			11,4	50,0	1,64	3,90		x				x
				26,0	114,4			13,0	57,2	1,87	4,46		x				x

***Table 46**. Estimated healthy wine dose and % gastrointestinal absorption for Women: 7.2% for phenols and 3.7% for anthocyanins. Results are presented as mg/day in three groups: < 1mg/day (low), 1<x<2 mg/day (medium) and > 2mg/day (optimum).*

		TOT. PRO mg/L	TOT. ANT. mg/L	PRO. TOT. Intake mg/day	ANT. TOT. Intake mg/day	% BIO. PRO	% BIO. ANT	PRO. diary intake %	ANT. diary intake %	PRO. EXP. serum intake mg/day	ANT. EXP serum intake mg/day	Serum PRO. EXP. Intake			Serum ANT. EXP. Intake		
												<1	1<x<2	>2	<1	1<x<2	>2
Women	Site 1	109,9	251,1	8,6	19,6	7,2	3,9	4,3	9,8	0,62	0,76	x			x		
				12,9	29,4			6,4	14,7	0,93	1,15	x				x	
				17,1	39,2			8,6	19,6	1,23	1,53		x			x	
	Site 2	84,4	345	6,9	28,3			3,5	14,1	0,50	1,10	x				x	
				10,4	42,4			5,2	21,2	0,75	1,65	x				x	
				13,8	56,6			6,9	28,3	1,00	2,21		x				x
	E (Mean Site 1 and Site 2)	97,2	298,1	7,7	23,9			3,9	12,0	0,56	0,93	x			x		
				11,6	35,9			5,8	18,0	0,84	1,40	x				x	
				15,5	47,9			7,7	23,9	1,12	1,87		x			x	
	Site 3	74,9	347,6	6,3	29,2			3,1	14,6	0,45	1,14	x				x	
				9,5	44,1			4,8	22,1	0,68	1,72	x				x	
				12,7	58,7			6,3	29,4	0,91	2,29	x					x
	Site 4	86,2	319,1	7,4	27,4			3,7	13,7	0,53	1,07	x				x	
				11,1	41,2			5,6	20,6	0,80	1,61	x				x	
				14,8	54,9			7,4	27,4	1,07	2,14		x				x
	Site 5	70,3	355,5	5,7	28,8			2,8	14,4	0,41	1,12	x				x	
				8,6	43,4			4,3	21,7	0,62	1,69	x				x	
				11,4	57,6			5,7	28,8	0,82	2,25	x					x
	L (Mean Site 1 and Site 2)	77,1	340,7	6,5	28,5			3,2	14,2	0,47	1,11	x				x	
				9,7	42,9			4,9	21,4	0,70	1,67	x				x	
				13,0	57,1			6,5	28,5	0,93	2,23	x					x

Data from **Table 45** and **Table 46** suggest that wines contribute to 2.9%–17.1% of daily total procyanidin and 9.8%–57.7% of daily total anthocyanin. Wines contribute significantly to total polyphenol intake (200 mg/day). While men ingested between 1.0 and 2.0 mg/day of procyanidins with all the estate wines, women could only achieve this concentration in three groups (Site 1, Site 2, and Site 4). The concentration in men reached > 2 mg/day for three of the estate wines (Site 1, Site 2 and Site 4), with higher procyanidin contents. On the other hand, if we consider the estate wine/dose, men only obtained > 2 mg/day procyanidin with 35 and 40 g Site 1, 40 g Site 2 and 40 g Site 4. In this case, men need to consume higher doses of estate wine to acquire > 2 mg procyanidins/day. Women could not obtain > 2 mg/day in all cases. In terms of anthocyanin composition in serum for women, the values depended on estate wine and estate wine/dose, while men achieved >2 mg/day with all estate wines. Women achieved a concentration of <2 mg/day anthocyanin with Site 1 estate wine and > 2 mg/day with the other wines (Site 2, Site 3, Site 4 and Site 5), though intake of higher volumes of wine.

Estate wine located in El Molar (Site 1) contained the highest tannin concentration (109.9 mg/L), lowest anthocyanin amount (251.1 mg/L) and highest degree of alcohol (16.1% alc. vol) relative to other wines (**Table 46**). The second highest level of procyanidin (86.2 mg/L) but lower alcohol (14.5%) was recorded for Site 4, compared to the other wines. Taking into account the influence of mesoclimate and grouping of wines as early region (E) and late region (L), no differences in total amounts were observed. Overall, > 2 mg/day procyanidin and anthocyanin contents were detected in men and $1<x<2$ mg/day in women for both E and L groups.

6.11 Conclusions

Although the bioavailability of wine-derived polyphenols in organisms is very low, polyphenols are associated with the beneficial effects of wine on human health. The type of wine with polyphenol compositions that may have greater beneficial effects on health considering a healthy recommended intake level remains to be established. Traceability of food, especially given the value of Estate wines, associated to single vineyards, is an important goal, allowing determination of the accurate composition of food in daily meals. Polyphenol intake for each individual depends on the total content of these substances in ingested food. In the case of wine, the total recommended amount for healthy individuals has limits due to the toxic effect of alcohol consumption. In this chapter we evaluated the beneficial effects of different ranges of recommended intake for wine (considered healthy) depending on gender and age. Both women and men were theoretically evaluated owing to different recommended healthy dietary levels of intake. Our main objective was to establish the influence of different estate wine compositions on estimated polyphenol consumption in humans. For this study, we comprehensively analysed five Priorat estate wines to ascertain how different intake levels lead to specific polyphenol patterns in serum.

The influence of altitude and sun exposure on polyphenol composition of the five Estate wines was additionally considered. Furthermore, the caloric effect of alcohol was calculated and different wine doses considered to determine the effect of the alcoholic degree of each wine on total calories in the diet due to wine consumption.

Considering the low absorption of phenolic compounds based on the previous publications, we hypothesized that some Estate wines provide similar polyphenol levels in serum of healthy individuals upon intake of a recommended amount on a daily basis. The choice of a Estate wine may avoid the effects of an elevated alcoholic degree. In this theoretical study, the caloric effect of alcohol was between 210 and 280 kcal for men and 70 and 140 kcal for women in relation to total calorie requirements (2366 kcal for men and 2078 kcal for women). This is a significant factor for consideration, since alcohol-derived calories represent 8.9% and 11.8% (minimum and maximum, respectively) of the total calorie requirement for men and 3.4% and 6.7% for women. Site 1, Site 2 and Site 4 estate wines provided higher amounts of procyanidins at a greater alcohol concentration (40 g) in both men and women, but Site 4 wine provided a lower concentration of alcohol. The total alcohol intake and total calorie count could be reduced without significantly affecting the amount of polyphenols acquired from each wine. All higher Estate wines/dose (Site 2, Site 3, Site 4 and Site 5) provided higher concentrations of serum anthocyanin but Site 4 estate wine was concomitantly associated with a lower alcohol content.

In summary, it is suggested that depending on the healthy recommended servings of wine for either women or men, different Estate wines can be selected to obtain similar amounts of procyanidins and anthocyanins in the diet. In addition, selection of specific Estate wines may avoid additional alcohol intake. This finding is of great importance because alcohol-derived energy is not usually considered in total energy requirements, which is essential for patients with obesity or diabetes who need to control weight and energy expenditure due to carbohydrates, respectively. Thus, even when the vineyards are relatively close in location, the precise geographical origin of the wine must be carefully considered during selection for daily intake. In this study, the mesoclimate of wine origin did not affect the total procyanidin and anthocyanidin range of concentrations (both geographically different areas, E (early) and L (Late)) but significant differences were observed among Estate wines. In view of recent reports, it can be further suggested that synergy of wine with other specific foods could facilitate gastrointestinal absorption and improve health benefits due to distinct wine compositions (for instance, combining pasta and wine instead of meat and wine). Although our present findings support the beneficial effects of moderate alcohol consumption, it is important to consider the high toxicity of alcohol and dependence when consumed in large quantities in a non-negligible percentage of individuals. We therefore do not recommend alcohol consumption by non-drinkers due to the risk of triggering an adverse situation of dependence or excessive consumption. Moderate alcohol drinkers should be advised to continue this habit without exceeding the

dose considered healthy (up to 30–40 g/day in men and 10–20 g in women), given no medical contraindications exist. Our preliminary research serves as a basis for future interventional studies to accurately evaluate the effects of wine produced from specific grape-growing areas on human health. As wine bottles do not show the composition, more complete labelling of wine showing the amount of total phenolic concentration would add an extra value together with the alcohol content.

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Chapter 7. Overall Discussion and Conclusions

The orography of the Priorat determines the particular mesoclimate of the region. Climatic parameters such as temperatures, rainfall and vapour pressure deficit show differences between nearby localities. The location of the vineyard plots, the altitude, the dominance of the humid sea breeze -*garbinada*- or the dry winds -*serè*-, the inclination of the slopes and the exposure of the vineyards, they form a certain mesoclimate that exerts a decisive influence on the ripening of the grapes. The effect of climate on phenology, potentially attributable to mesoclimatic variations, results in greater variability in budbreak and veraison dates that depends on previous recorded temperatures. A temperate vintage delays budbreak when compared with a warm vintage. However, in a temperate vintage, the differences are less noticeable at the beginning of bud break and veraison between the early and late regions. Moreover, the extended summer at the end of the ripening period causes the harvest date to advance 15 days. A delay in bud break do not result in a delay at harvest; warm years in the late region resulted in an earlier harvest date. These observations are associated with high temperatures occurring in late August and even September in the Priorat, which results in accelerated grape ripening.

The climatic variability in the same vintage manifests significantly during the maturation period and is determined by the increase in temperature and the deficit in vapor pressure. From weather records, the late ripening region resulted in lower temperatures, vapour pressure deficit and growing degree days. Moreover, climatic data revealed 2009 and 2011 as a warmer vintage. The persistence during the ripening season around harvest of high VPD values gives rise to notable inter-plot differences in warm years and early areas. In general, plots with higher vegetative growth and lower production, would be more vulnerable to climate change, less predictable in relation to the composition of the grape and wine, and with variability in the evolution of the grape composition during ripeness. The type of soil and the existing drainage in terraces and slopes determines a variability in production per vine. Recent studies in Penedès show that water retention in the soil will be crutial to fulfill the water needs as well as the agronomic practices with the maintenance of enough organic matter into the soil (Funes et al., 2020). What is more, using precision viticulture tecniques (e.g use of NDVI, soil variability maps, sensoring the vineyards in a geostatistical approach to delineate irrigation management zones and to identify the most representative spots within a field for point measurement) as well as regenerative agriculture techniques will help to give a quicker response to an unexpected weather event.

It can be suggested that the cultivar Grenache responds more quickly to environmental changes when water stress conditions are more extreme. Grenache may behave by showing a stomatal closure during periods of high VPD and thus avoiding a large loss of water, recovering at times of day that are more favourable. In the opposite direction,

Carignan show more negative water potentials, a stomatal opening and therefore a more constant water loss. Previous research carried out in grapevines show that water stress greatly reduces the hydraulic conductivity (Kh) of the xylem (Lovisolo and Schubert, 1998; Schultz, 2003; Alsina et al., 2007) and it is determined by the anatomical features of the xylem through apoplastic transport (Tyree, 2003; de Herralde et al., 2006), or by the symplastic and transcellular pathways (Tyerman et al., 2002), possibly through aquaporins (Vandeleur et al., 2009). Along with these restrictions on general circulation, it has now been shown that the hydraulic conductivity of the leaf is generally responsible for at least 30% of the loss of hydraulic conductivity under stress (Sack and Holbrook, 2006), and that this reduction presents strong interspecific variability often related to petiole morphology and vessel architecture (Charrier et al., 2018). Stomatal density and size are responsive to environmental conditions during leaf development and are invariant after the leaf is fully expanded (Düring, 1980, Rogiers et al., 2011). In contrast, the size of the pore opening is adjusted reversibly in response to the environment internal and external to the plant (Aphalo and Jarvis, 1991, Cowan,1977). The responsiveness of stomata to the environment hence results from the combination of both invariant and reversible responses operating at different time scales (Bresta et al., 2011).

Additionally, Grenache and Carignan have very different leaf morphologies from an ampelographic point of view. In fact Gago et al., (2019) found that Grenache compared to Syrah had a significantly smaller leaf surface area, but a significantly thicker leaf blade. It also had significantly larger stomata and a larger stomatal index than Syrah. The distribution of mesophyll tissues was similar in both cultivars, but the upper epidermis was significantly thicker in Grenache Noir, and the palisade parenchyma cells were longer in Syrah. Further work could be done amongst Grenache and Carignan that are needed to determine how these morphological differences may be connected with different responses at the functional level.

Even if Grenache showed more isohydric behaviour compared to Carignan, under episodes of unexpected heat and drought, the response of the plant tends towards survival by closing the stomata in both varieties. It is also possible that a combination of both signals - hydraulic and hormonal -, such as hydraulic conductance (kh) and ABA, allows some species to change from isohydric to anisohydric behaviour (Domec and Johnson, 2012), depending on the circumstances of the environment. It should be noted that the behaviour that the same variety can show in different mesoclimate within the same region during an experiment is subject to several conditions that modify this behaviour (Medrano et al., 2003; Williams and Baeza, 2007 ; Chalmers, 2007; Poni et al., 2007; Santesteban et al., 2009; Lovisolo et al., 2010; Chaves et al., 2010; Collins et al., 2010; Rogiers et al., 2011; Pou et al. ., 2012). The classification of isohydry and anisohydry for species and cultivars is dependent on many factors including water potential regulation, stomatal behaviour, and hydraulic transport under drought conditions (Martínez-Vilalta and Garcia-Forner, 2016; Dal Santo et al., 2016). A recent meta-analysis examined factors

influencing stomatal conductance in grapevine in response to water availability proposing that there is a continuum of stomatal responses that are dependent upon the scion – rootstock combination and the interaction with different soil types (Lavoie-Lamoureux et al., 2017).

The precocity of the plot determines the kinetics of maturation in areas with a variable orography and affects the composition of the pulp, causing a disjunction between the maturity of the pulp and the phenolic maturity of the skin. The results of this study indicate that the plots located in precocious areas and on stony soils present greater vulnerability in vintages with severe climate and drought, showing, at the end of maturation, a significant increase in sugars that does not correspond to the consequent anthocyanin concentration in berry. It is well known that the optimum temperature for anthocyanin synthesis is around 30ºC, and that higher values up to 35ºC inhibit it. Therefore, modification of the vine microclimate through canopy management can prevent excessive sunlight and high temperatures from reaching the bunch and improve anthocyanin content (Downey et al., 2003; Tarara et al., 2008). Also the severity of leaf removal should be taken into account as it affects the enological parameters of grape must, particularly sugars, acidity and aminoacidic composition (Yue et al., 2019; Zhang et al., 2017).

Particularly, in earlier areas, the rate of acid degradation and accumulation of sugars takes place from earlier stages. However, the synthesis of anthocyanins and their accumulation varies depending on vintage. Anthocyanins reach higher concentrations in the early zone favored by temperate vintages, on the other hand, in plots of late zones in altitude the accumulation of these compounds decreases. In warm years, grapes in the early mesoclimate reach a higher alcoholic degree than in the late, while the acidity is higher in the late mesoclimate. Anthocyanins show variability in terms of vintage and plot, whereas the accumulation of tannins is more notable in grapes from early and warm regions. In the temperate year 2010, the anthocyanin concentration did not vary between mesoclimates. Summarizing, the knowledge of the kinetics of evolution of the quality compounds of the berry and in particular, the evolution of phenolic compounds according to the topography/mesoclimate of the plot, will be of great importance in viticulture of terraces and slopes to determine the vintage date for obtaining quality wines.The prediction of an interval of concentrations of anthocyanins and tannins is of utmost importance to define the styles of wine and at the same time adapting the techniques of extraction of phenols in the winery to achieve the appropriate tanicity in the wines produced.

To sum up, oenologists have the challenge to understand the physiology of the grapevines Grenache and Carignan to better assess the water stress and the implication on the accumulation of colour and tanins. As an example, DOQ Priorat grape production in 2019 decreased in 1.1 milion due to unexpected heat event during the last week of June. Understanding the mechanism of the vines to cope with the unexpected climate effects that are becoming more frequent, together with implementing precision viticulture and

adapting the techniques of extraction of color and tannin during fermentation will have to be managed with greater knowledge and more precisely, as well as deciding which is the best time of harvest, depending on the water status of the plant.

The Greek physician Hippocrates considered wine a part of a healthy diet. The future consumtion of wine, whether it turns out to be, will not be just one thing. It will be the combinaton of what happens on the politics, the economics, the public health, the environment but the attention should be focused in consuming moderate amounts of wine under a Mediterranean diet. Recommendations of wine consumption should be always related to the composition and always avoiding heavy drinking. A more personalised standar drinking amount should be considered.

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Received: 23 August 2020 | Revised: 7 March 2021 | Accepted: 10 March 2021
DOI: 10.1002/pld3.319

ORIGINAL RESEARCH

Water stress assessment on grapevines by using classification and regression trees

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Funding information
This research was funded by the University Rovira i Virgili, Biochemistry Department, Viticulture Research Group. Partial support for this work has been received from the Agency for Management of University and Research Grants (AGAUR) of the Government of Catalonia (grant 2017 SGR 705).

Abstract

Multiple factors, such as the vineyard environment and winemaking practices, are known to affect the development of vines as well as the final composition of grapes. Water stress promotes the synthesis of phenols and is associated with grape quality as long as it does not inhibit production. To identify the key parameters for managing water stress and grape quality, multivariate statistical analysis is essential. Classification and regression trees are methods for constructing prediction models from data, especially when data are complex and when constructing a single global model is difficult and models are challenging to interpret. The models were obtained by recursively partitioning the data space and fitting a simple prediction model within each partition. The partitioning can be represented graphically as a decision tree. This approach permitted the most decisive variables for predicting the most vulnerable vineyards and wine quality parameters associated with water stress. In Priorat AOC, Carignan grapevines had the highest water potential and abscisic acid concentration in the early growth plant stages and permitted vineyards to be classified by mesoclimate. This information is useful for identifying which measurements could most easily differentiate between early and late-ripening vineyards. LWP and T_s during an early physiological stage (pea size) permitted warm and cold areas to be differentiated.

KEYWORDS
ABA, anisohydric, carignan, classification and regression trees, isohydric, water stress

1 | INTRODUCTION

Water stress on vine plants induces the synthesis of secondary metabolism. Around veraison, water deficit stress causes a significant increase in the abscisic acid (ABA) level in fruit zone leaves (Okamoto et al., 2004) and berries (Coombe & Hale, 1973; Düring & Allenweldt, 1980). ABA plays an important role in the regulation of growth and the ripening of vines. Lack of water in the soil and elevated temperatures induce the synthesis of ABA in the roots, followed by its translocation to the leaves, where it rapidly alters the osmotic potential of stomatal guard cells, causing them to shrink and the stomata to close. Stomatal closure reduces transpiration and thus prevents further water loss from the leaves during periods of low water availability. Around veraison, ABA levels in grapes

One-sentence summary: Classification and regression trees is a very easy-to-use statistical tool for vineyard parameters characterization from high variability data.

increase significantly, along with the stimulation of ripening and phenolic synthesis, but decrease during the final stage of berry ripening (Bondada & Shutthanandan, 2012; Palejwala et al., 1985; Soar et al., 2006; Wheeler et al., 2009). Abscisic acid may be translocated from the sites of biosynthesis, such as roots and leaf vascular tissues, to the guard cells. Recent identification of multiple transmembrane ABA transporters indicates that the movement of this hormone within plants is actively regulated in an intercellular network (Kuromori et al., 2018).

Regulation of water deficits has often been used to balance grapevine vegetative and reproductive growth to control berry quality (Chaves et al., 2010). Analysis of the phenolic composition in wine is essential for establishing quality parameters related to water stress, as some studies have shown that ABA is involved in the mechanisms controlling the synthesis of anthocyanins and promotes the synthesis of tannins accumulating in skin (Lacampagne et al., 2010) ABA synthesis depends on different factors promoting water stress; plant water physiology is affected by various environmental factors (e.g., topography, soil water-holding capacity, temperature, rainfall, and vapor deficit pressure), plant vigor, and cultural practices, such as irrigation techniques and fertilization programs (Downey et al., 2004; Jackson & Lombard, 1993) and by scion/rootstock interaction with soil type (Lavoie-Lamoureux et al., 2017), Grenache is highly influenced by vigor, because anthocyanin accumulation is favored in balanced, high-vigor vines, whereas in Carignan, the anthocyanin content varies under the combined effects of vigor, rootstock, berry size, and vintage (Edo et al., 2014).

Appropriate statistical tools are required for identifying the factors that have the strongest effects on quality and stress during growth (plant) and maturation (grape). Predictors, such as linear or polynomial regressions, are global models, where a single predictive formula is applied over the entire dataset. However, when the data interact in complex, nonlinear ways, assembling a single global model is challenging. Classification-type problems can be resolved when a categorical dependent variable (e.g., class and group membership) is predicted from one or more continuous and/or categorical predictor variables. Generally, the purpose of analyses involving tree-building algorithms is to determine a set of *if–then* logical (split) conditions that permit accurate prediction or classification of the data.

The aim of this study was to evaluate the efficacy of a multivariate nonparametric technique of classification and regression trees (CART) for identifying and selecting the most important factors affecting water stress in vineyards with a heterogenic orography (e.g., leaf water potential [LWP], concentration of ABA, surface leaf temperature [T_s]); analyze the effect of these interactions on final grape and wine quality (e.g., composition of anthocyanins and procyanidins); and improve the rapidity with which ABA can be measured in grapevine leaves. The heterogeneity of the vineyards in the Priorat wine region requires the collection of a considerable amount of data and more robust statistical tools to better understand the factors affecting water stress in vineyards. Because of the increasing drought and higher temperatures occurring in the Priorat, the Priorat is highly vulnerable to future climate change. Here, we explore applications of multivariate nonparametric classification techniques such as CART, a type of decision tree technique (Breiman et al., 1984), given that traditional methods are not appropriate for analyses because of the characteristics of the variables studied.

TABLE 1 Values of predawn leaf water potential (PLWP, Ψ_{PLWP} [MPa]) and midday leaf water potential (MLWP, Ψ_{MLWP} [MPa]) for Sites 1, 2, 3, 4, and 5 at two different stages of growth—pea size (PS) and veraison (V)—at predawn and midday

	Pea size (PS)		Veraison (V)	
Site	Ψ_{PLWP} Predawn	Ψ_{MLWP} Midday	Ψ_{PLWP} Predawn	Ψ_{MLWP} Midday
1	−0.33 (0.04)	−1.29 (0.05)	−0.47 (0.12)	−1.38 (0.07)
2	−0.43 (0.08)	−1.21 (0.16)	−0.54 (0.13)	−1.44 (0.08)
3	−1.43 (0.01)	−1.48 (0.04)	−0.82 (0.21)	−1.76 (0.07)
4	−1.27 (0.04)	−1.39 (0.05)	−0.47 (0.05)	−1.58 (0.06)
5	−1.28 (0.03)	−1.50 (0.00)	−0.92 (0.08)	−1.50 (0.04)

Note: Values are mean and standard deviation.

TABLE 2 Values for abscisic acid concentration (ABA) for Sites 1, 2, 3, 4, and 5 at two different stages of growth—pea size (PS) and veraison (V)—at predawn and midday

	Pea size (PS)		Veraison (V)	
Site	[ABA] Predawn	[ABA] Midday	[ABA] Predawn	[ABA] Midday
1	152.8 (4.7)	195.0 (33.4)	162.8 (7.6)	243.5 (13.1)
2	181.1 (21.4)	226.4 (5.9)	92.5 (8.7)	115.5 (3.6)
3	152.0 (17.3)	229.0 (42.2)	97.3 (15.5)	89.9 (8.6)
4	211.8 (5.5)	423.0 (80.7)	83.7 (2.4)	134.8 (38.7)
5	196.3 (5.9)	400.1 (19.8)	114.9 (12.7)	178.8 (9.3)

Note: Mean and standard deviation.

2 | RESULTS

2.1 | LWP and ABA

LWP and ABA measurements are shown in Tables 1 and 2. After characterizing differences in variability through a nonparametric Kruskal–Wallis test (Table 3) at a significance level of 5%, Pearson correlations between the measured variables and their significance (Table 4) were calculated. The classification of sites was captured by the Classification and Regression Trees (CART) to help identifying key variables in the data. The most meaningful predictors were used to create the tree. Plant, grape and wine data were collected to evaluate the interactions. However, to obtain reliable classification and regression trees, a previous selection of child nodes was completed using the easiest-to-measure variables in the field and the easiest-to-analyze variables in the laboratory. Each round of data is known as 'nodes'. Each node will have an *if–else* clause based on a labeled variable. Based on that question each instance

TABLE 3 Analysis of the differences between groups using the nonparametric Kruskal–Wallis test

Conditions	Hour	Phenological stage	*p* value
Leaf water potential	Predawn	PS	.014
	Midday	PS	.014
	Midday	V	.017
Abscisic acid content	Predawn	PS	.019
	Midday	V	.017
Leaf surface temperature	Predawn	PS	.012
Total anthocyanins		Wine	.019
Glycosylated anthocyanins		Wine	.014
Acetyl glycosylated anthocyanins		Wine	.011
Berry weight			.009
Total leaf area/kg		V	.024
		RP	.019

Abbreviations: PS, pea size; RP, ripeness; V, veraison.

of input will be directed/routed to a specific leaf-node which will tell the final prediction. The tree depth is chosen as the most number of levels desired in the decision tree. The first node is split based on the most important predictor, then the following child nodes are broken down to separate out the next variable. Entering a value, the program sets the minimum number of cases an internal node is to be split. Three times terminal node limits allow a reasonable number of splitters.

2.1.1 | CART: WATER STRESS AND PLANT GROWTH

Plant growth parameters that differed significantly (p value ≤ .05) between plots were berry weight and total leaf area/kg (TLA/kg) at the veraison (V) and ripening (RP) stages. Water stress indicators that differed significantly between plots were LWP and [ABA] at pea size (PS) and veraison(V) and surface temperature (T_s) at pea size (PS). Pearson correlations revealed that LWP at PS measured at 8:00, ABA at V measured at 14:00, and T_s at PS measured at 8:00 were negatively correlated with the synthesis of anthocyanins in wine for all anthocyanin families (acylated and non-acylated). LWP and T_s showed stronger correlations when these parameters were measured earlier in the day (8:00) or at the beginning of the vegetative cycle (PS). The same variables—LWP at PS measured at 8:00, ABA at V measured at 14:00, and T_s at PS measured at 8:00—were positively correlated with TLA/kg V.

As a result from this the CART, LWP at PS measured at 8:00 was the most important predictor allowing to create the first node that separated early mesoclimates (Nodes 6 and 7) from late mesoclimates (Nodes 4 and 5). Nodes 2 and 3 were dependent on

TABLE 4 Pearson correlation matrix

Pearson correlation matrix	[ABA] (ng/g) predawn PS	[ABA] (ng/g) midday V	Ψ_{PLWP} (MPa) predawn PS	Ψ_{MLWP} (MPa) midday PS	Ψ_{MLWP} (MPa) midday V	Ts (°C) predawn PS	Berry weight (g)	TLA/kg V	TLA/kg RP	ANT (mg/L) wine	A-G (mg/L) wine	A-AG (mg/L) wine
[ABA] predawn PS	**1**											
[ABA] midday V	−0.143	**1**										
Ψ predawn PS	−0.283	0.489	**1**									
Ψ midday PS	−0.011	0.145	**0.798**	**1**								
Ψ midday V	0.116	**0.696**	**0.796**	**0.574**	**1**							
Ts predawn PS	−0.226	0.451	**0.935**	**0.763**	**0.702**	**1**						
Berry weight	−0.228	0.316	**0.783**	**0.697**	0.492	**0.917**	**1**					
TLA/kg V	**−0.516**	**0.627**	**0.738**	0.437	**0.578**	**0.703**	**0.566**	**1**				
TLA/kg RP	−0.304	**0.682**	0.015	−0.262	0.190	−0.006	−0.161	0.478	**1**			
ANT-wine	0.319	**−0.703**	**−0.588**	−0.408	−0.461	**−0.737**	**−0.752**	**−0.699**	−0.451	**1**		
A-G-wine	0.278	**−0.680**	**−0.576**	−0.380	−0.461	**−0.752**	**−0.776**	**−0.661**	−0.407	**0.986**	**1**	
A-AG-wine	**0.610**	**−0.628**	**−0.658**	−0.411	−0.393	**−0.732**	**−0.738**	**−0.846**	−0.449	**0.917**	**0.882**	**1**

Note: Bold values are different from 0 at a significance level (α) of 0.05.

Abbreviations: A-AG, acetyl glycosylated anthocyanins; ABA, abscisic acid; A-G, glycosylated anthocyanins; ANT, total anthocyanins; PS, pea size; RP, ripeness; TLA, total leaf area; T_s, leaf surface temperature; V, arount veraison; Ψ_{MLWP}, midday leaf water potential; Ψ_{PLWP}, predawn leaf water potential.

ABA at PS (late mesoclimate) and V (early mesoclimate). However, obtaining a partition of the five sites [ABA] at V was decisive and resulted in the generation of Nodes 8 and 9. As a consequence, the sites with the highest probability of being classified with LWP values ≤−0.863 (8:00 at PS) were the parcels located in the town of Molar (Sites 1 and 2). Hence, Site 1 had levels ≥ABA 175.9 ng/g (14:00 at V) (Figure 1). Site 3, within the late mesoclimate area, had a lower probability of having ABA ≤ 183.9 ng/g (morning at pea size) PS. Fewer factors differentiated Site 3 (gray) from the other sites; it was thus separated in an early node as in Sites 1 and 2 (blue and red) of the early mesoclimate area (Figure 1).

2.1.2 | CART: ABA, LWT, AND T_s

The most significant variables for characterizing and classifying the observations were [ABA], LWP, and T_s. T_s was selected given that it had a direct relationship (positive Pearson correlation) with the vegetative growth parameters of TLA/kg and berry size. The Pearson correlation produced a clear classification tree (Figure 2) based on the T_s, at the root node, it generated three child nodes (2, 3, and 4). This first classification by T_s at PS measured at 7:00 resulted in a purity of 100% for Site 4, but the T_s at PS measured at 12:00 was clearly the most important variable for Sites 5 and 6 under a second child node classification. However, the early sites (1 and 2) were differentiated by [ABA] at PS measured at 8:00.

Although many authors have described the effect of T_s on the quality of grapes during the ripeness period (Greer & Weedon, 2013; Spayd et al., 2002; Van Leeuwen et al., 2009), the analysis of the tree shows the magnitude of the effect of T_s from the early stage of PS. Measurements taken at 8:00 at PS were more likely to have values of T_s ≤ 22.0°C in the late mesoclimate area. Child Node 4 indicates that Sites 1 and 2 had a high probability of being classified within the temperature range 22°C ≤ T_s ≤ 24°C (8:00 at PS). Using the CART greatly facilitates the characterization of the importance of the classification of vineyards, especially in the late area (Sites 3, 4, and 5). Furthermore, Sites 3 and 5 were located in equivalent positions in the tree (purity 50%); thus, the differentiation of both plots from other plots depended on the same factors. Remarkably, both Site 3 and Site 5 had similar TLA and thus greater water loss. (Figure 2).

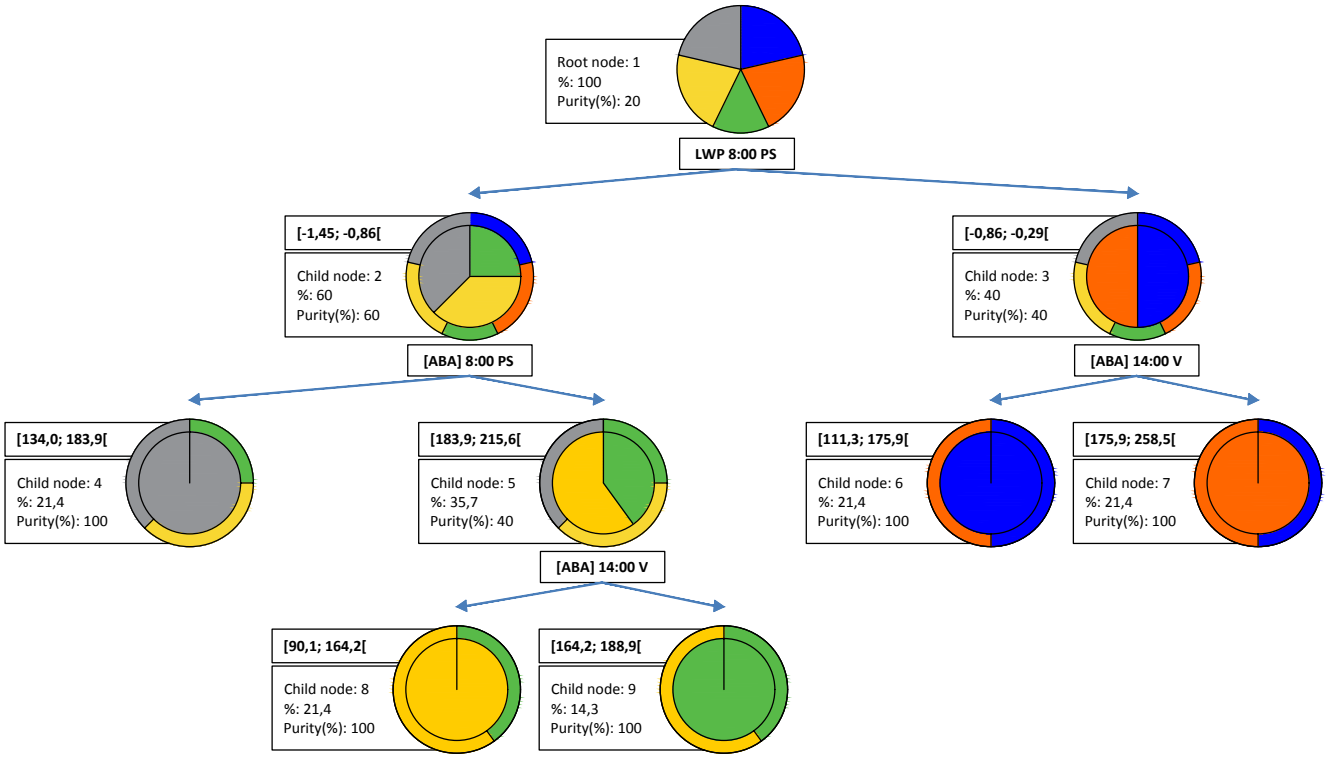

FIGURE 1 Classification and regression trees by water stress indicators (LWP, ABA, and T_s). Site 1 (red), Site 2 (blue), Site 3 (gray), Site 4 (orange), and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child nodes are broken down to separate out the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each color represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the *if–else* question. The circles on the right branch correspond to those vineyards with higher values and those on the left to those with lower values, in answer to the *if–else* question (values are shown in brackets)

2.1.3 | CART: ANTHOCYANINS IN WINE QUALITY

In this CART analysis, Pearson correlations of plant parameters and wine composition in each site were calculated. Both LWP at PS measured at 8:00 and LWP at V measured at 14:30 were correlated with ANT (mg/L), A-G (mg/L), and A-AG (mg/L). However, lower correlation coefficient values were obtained for LWP at V measured at 14:30 pm. Despite the difficulty of establishing direct links between plant parameters (TLA/kg at V) and wine composition (anthocyanins), robust correlations were found for T_s at PS measured at 7:00 and wine anthocyanins (non-acylated and acylated). The most significant relationship was for the correlation between TLA/kg V and A-AG (mg/L).

Based on the easy-to-measure parameters in the vineyard, such as T_s and the ratio of leaf area and production at V (TLA/kg V), we could characterize the relationship between the water status of plants and plant growth to the quality of the final wine product. This classification of plots allowed us to determine patterns of heterogeneity between plots. Thus, the CART classifies sites through the nodes to distinguish among different vineyards. (Figure 3).

The tree shows that LWP (Node 1) at PS permitted the differentiation of early (EM) and late (PO) sites. Values within the range $-1.45 \leq \text{LWP} \leq -0.862$ described the late ripeness sites (4, 5, and 6), while the range $-0.863 \leq \text{LWP} \leq -0.290$ classified the warmest sites (1 and 2). In the late mesoclimate area (Node 2), sites were separated by anthocyanins; Sites 3, 4, and 5 were classified together by Node 5 and were primarily influenced by the LWP at 14:30 in V. This finding suggests that the topography of the vineyard location, as well as the climate and soil type, had an important influence on wine quality. However, the parameter that classifies vineyards was ABA at 14:00 V (≤175.9 ng/g) by Node 3 and was necessary for divided Sites 1 and 2 (early mesoclimate). Thus, LWP did not affect the phenolic content of the wines.

3 | DISCUSSION

Measurements of the distribution of soil water revealed that the differences detected among the five sites reflected heterogeneity in soil particle size, depth, and texture. Sites 1 and 2 (El Molar) on a clayey soil had a higher water-holding capacity, than that of Sites 3, 4, and 5 (Porrera), which were steep with more stones and soil was primarily composed of larger elements. Thus, the vines in the town of El Molar (Sites 1 and 2, early mesoclimate) had more available water than those in Porrera (Sites 3, 4, and 5, late mesoclimate), despite the lower rainfall recorded during the cycle. Predawn leaf water potential (PLWP) reflects soil water availability as perceived by the plant and midday leaf water potential (MLWP) measures leaf water potential under maximum daily water demand. Therefore the higher soil water content at Site 1 and Site 2 led to more vigorous plants because LWPs were less negative. In Porrera, because of the lower water retention in soils, the plants had more negative LWPs than those in Molar. In addition, at approximately the pea size phenological stage, water transpiration by leaves was higher and LWPs showed more negative values because of the low soil water

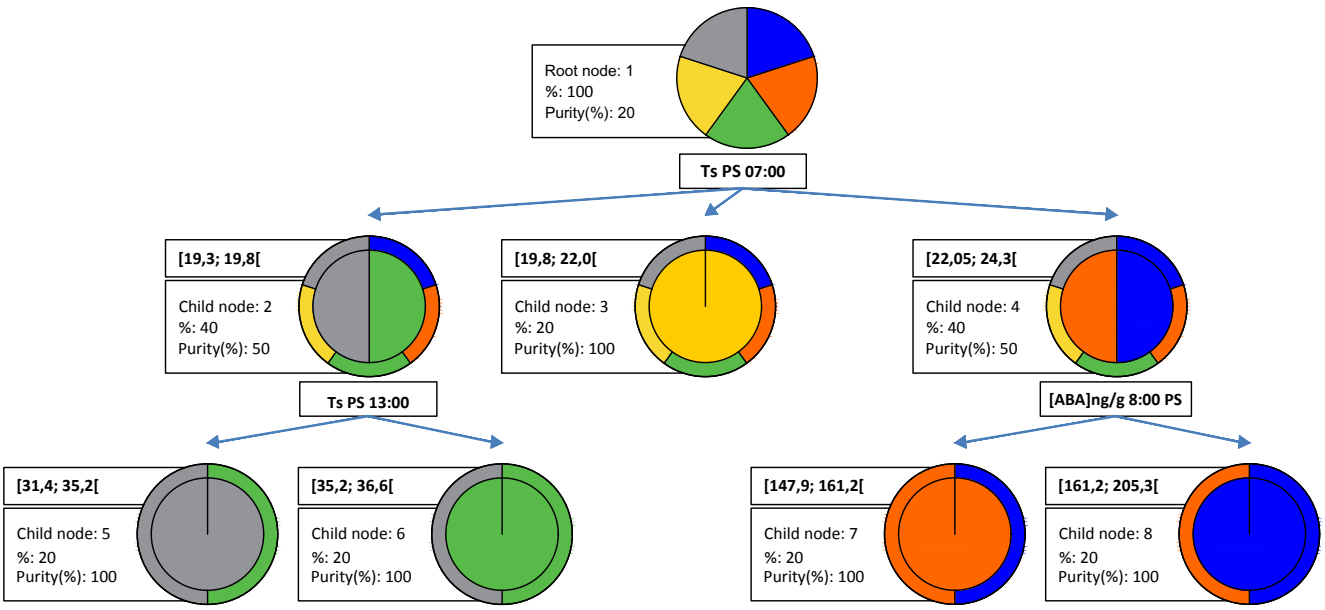

FIGURE 2 Classification and regression trees by T_s (surface canopy temperature). Site 1 (red), Site 2 (blue), Site 3 (gray), Site 4 (orange), and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child nodes are broken down to separate out the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each color represents the data from a single vineyard. The inner circle is the percentage that results from answering the *if–else* question. The circles on the right branch correspond to those vineyards with higher values and those on the left to those with lower values, in answer to the *if–else* question (values are shown in brackets)

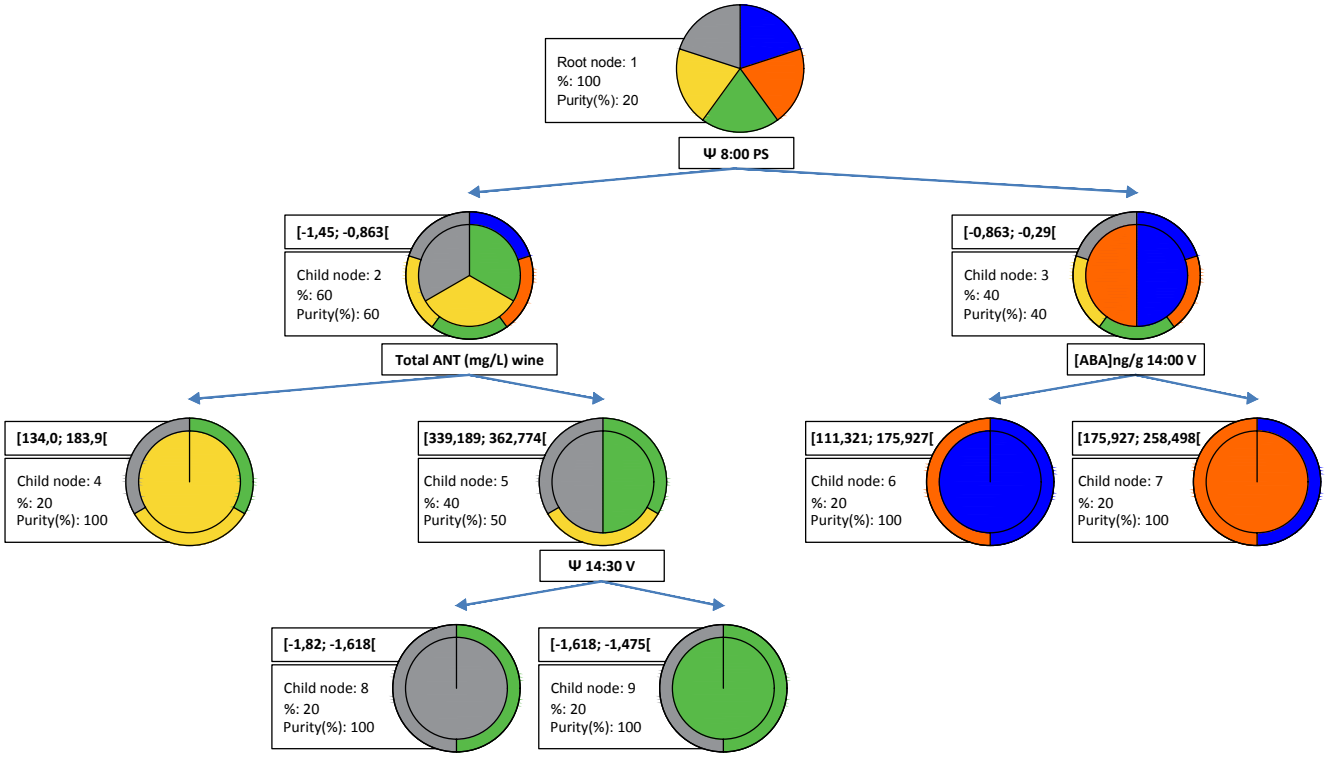

FIGURE 3 Classification and regression trees by total anthocyanins. Site 1 (red), Site 2 (blue), Site 3 (gray), Site 4 (orange), and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child nodes are broken down to separate out the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each color represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the *if–else* question. The circles on the right branch correspond to those vineyards with higher values; those on the left to those with lower values, in answer to the *if–else* question (values are shown in brackets)

content in the stony and poor soil. It is known that *Vitis* genotypes show either an isohydric or anisohydric response to water stress. In isohydric cultivars, strong control of stomatal conductance by ABA reduces transpiration, obviates decreases in water potential, and delays the onset of stress tolerance mechanisms. In contrast, weak ABA control of stomatal closure does not avoid midday decreases in water potential in anisohydric grapevines (Lovisolo et al., 2010). In addition, during periods of low water availability and higher transpiration water demand, many authors have observed that a hydraulic signal can also have a controlling effect on stomatal conductance, and this also relates to both patterns, isohydric species maintain relatively stable LWPs precisely because of their more strict stomatal control, whereas anisohydric species would show a looser regulation of transpiration. What is more, the degree of isohydry can be related to a reduced soil water availability (lower, more negative soil water potential, Ψ_{soil}) may affect plant conductance in two ways, by lowering its hydraulic conductance (K_H) and/or its leaf conductance (g_{Leaf}). These reductions, have opposite effects on the water potential difference through the plant ($\Delta\Psi = |\Psi_{Leaf} - \Psi_{soil}|$), whereas lower K_H increases $\Delta\Psi$, lower g_{Leaf} decreases $\Delta\Psi$ (Martínez-Vilalta & García-Forner, 2017; Martínez-Vilalta et al., 2014). Thus, there is a tight coordination between hydraulic and water vapor transport at the plant level (Sperry & Love, 2015).

Parameters that best discriminated between sites were LWP and ABA content, followed by berry size and anthocyanin concentration. Around veraison, higher correlations between LWP and ABA content were obtained. After analysis of the Pearson correlations, the best results were obtained for the veraison phenological stage where vapor pressure deficit (VPD) is lower. ABA concentrations in Carignan vines at different sites (early (1 and 2) and late (3, 4, and 5)) are shown in Table 4. Higher concentrations of ABA were observed in all vineyards when measurements were taken at noon. This observation reflects increased water stress in all plots and confirmed measurements of LWP. It also established a direct correlation between the concentration of ABA and LWP (R^2 = .918). The strongest correlations were observed for the first measurements in the morning, while measurements at noon showed greater dispersion, R^2 (.7175). Thus, the CART analysis could distinguish among sites of the later mesoclimate region based on ABA at pea size stage.

In Figure 1, PLWP at pea size separated sites within mesoclimate and reached values of −0.86 for the early and −1.45 for the late mesoclimate. Around veraison, ABA concentration classified vineyards in the warmest area with values of 258 ng/g in Site 1 and 175 ng/g in Site 2. In the coldest area, the values were lower and did not separate at such wide intervals. At Site 3, ABA concentration did not

exceed 183 ng/g at pea size; instead, values were higher in Sites 4 and 5 but did not differentiate vineyards. Values for these plots at veraison were lower than in pea size; Site 3 had values as high as 164 ng/g, and Site 5 had values as high as 188 ng/g. The three most similar sites in ABA at veraison at noon were Sites 2, 4, and 5. Thus, the ABA concentration at veraison is important for differentiating most of the plots, including Sites 1, 2, 4, and 5.

In Figure 2, T_s at pea size measured at predawn permitted separation by temperature ranges and isolated Site 2 with temperatures between 19.8°C and 22°C. The early sites were separated by ABA at pea size at predawn (with higher values in Site 2, considering that Site 1 had a rocky soil, while Site 2 was composed by finer elements). Plots of the coldest area were only separated by T_s at pea size at noon. Site 5 was located at higher elevation and experienced higher temperatures at noon (36.6°C) than Site 3 (35.2°C). T_s at veraison did not provide useful information because the plots experienced similar levels of stress. Thus, the characterization of the plots by T_s can be predicted at pea size but not around veraison.

In Figure 3, PLWP at pea size separated sites with different mesoclimates. Sites 1 and 2 differed in ABA around veraison at noon (Figure 1). The ABA concentration in Site 1 was twice that of Site 2; thus, these plots did not differ in the concentration of anthocyanins unlike the colder sites. Sites in the cold mesoclimate were classified by the anthocyanins in wine. Although there was a strong correlation with anthocyanins in grapes, wine correlated with other variables (as evidenced by Pearson correlations greater than 0.7). Plants at Site 2 were the least vigorous with anthocyanin values less than 339 m/L. Because plants at Sites 3 and 4 showed more vigor, the effect that distinguished the plots was MLWP at veraison, as the water stress was increased in Site 3 (LWP of −1.82) and Site 4 (LWP of −1.6).

Even if the action of ABA in occlusive cells is complex and not yet fully understood, *Vitis* genotypes apparently exhibit different levels of drought adaptation that differ in key steps involved in ABA metabolism and signaling (Rossdeutsch et al., 2016). In general, *Vitis vinifera* varieties, displayed more pronounced responses to water deficit in comparison to other *Vitis* genotypes. Moreover, Dal Santo et al., (2016) proposed a cause–effect link between the physiological grapevine plant conditions and the intensity of the gene expression changes. Finally, in regards to grape composition, many key genes (VvMybA1 and VvUFGT) of the flavonoid biosynthetic pathway are also up-regulated during ripening, resulting in a berry quality increase (Ferrandino & Lovisolo, 2014). ABA accumulation and the induction of flavonoid biosynthesis increase the quality of berries by facilitating the accumulation of secondary metabolites, especially polyphenols. Under water stress, polyphenolic concentrations increase in berries both in isohydric varieties, such as *Grenache* (Coipel et al., 2006), *Tempranillo* (Santesteban et al., 2011), *Manto negro* (Medrano et al., 2003), and in anhysohydric varieties, such as *Cabernet Sauvignon* (Bindon et al., 2008; Kennedy et al., 2002), *Cabernet Franc* (Matthews & Anderson, 1988), and *Muscat of Alexandria* (Dos Santos et al., 2007), with different temporal dynamics related to ABA induction. Aquaporins are another target for ABA to regulate both water and carbon fluxes. ABA affects aquaporin regulation in response to abiotic stresses (Kaldenhoff et al., 2008) by modulating their gene expression and protein abundance or activity, affecting in cellular water relations and cell metabolism in response to water stress. Aquaporins can be modulated at several levels, via transcription, translation, trafficking and gating (opening and closing of the pore) and by environmental and developmental factors (Chaumont & Tyerman, 2014), such as: irradiation (Lopez et al., 2013; Prado et al., 2013), transpiration (Laur & Hacke, 2013; Sakurai-Ishikawa et al., 2011), circadian rhythms (Hachez et al., 2008), abscisic acid (ABA) feeding (Pantin et al., 2013; Shatil-Cohen et al., 2011), auxin feeding (Péret et al., 2012) and shoot wounding (Sakurai-Ishikawa et al., 2011; Vandeleur et al., 2014). Coupled with that, Castellarin et al., (2007) showed that water stress favored the accumulation of more hydroxylated and methylated anthocyanins (peonidin 3-O-glucoside and malvidin 3-O-glucoside). In addition, the degradation of anthocyanin would probably be induced by high temperatures with an oxidative stress leading to the formation of H_2O_2, with the subsequent induction of peroxidases and of oxidoreduction enzymes (Mori et al., 2007). In contrast, little is known about the impact of temperature on proanthocyanidin accumulation in grape skins; berries are able to compensate the initial effects of temperature on proanthocyanidin biosynthesis resulting in similar concentration of proanthocyanidin at harvest (Cohen et al., 2012).

Overall, the effect of variables on the classification of the trees was closely tied to the water scarcity of the plants. In viticulture science it is of particular importance to evaluate whether the relationships between physiological parameters fitted to data through these powerful statistical methodologies. In addition, some authors (Brillante et al., 2017) have shown that well-trained machine-learning models can be used to capture the essential relationships between plant physiology and the environment. As an example, Brillante et al., (2016) have for the first time modeled grapevine water stress. This models will be important to design experiments and provide with validation tests to demonstrate the efficiency of the models.

4 | CONCLUSIONS

To assess water stress in grapevines, both LWP and concentration of ABA are important for characterizing the physiology of the growing season and its effects on phenol grape quality. A methodology that permits rapid and accurate responses to ABA to be determined, that indicates the water deficit, and that measures vegetative and productive growth (berry weight and TLA/kg) can help elucidate how periods of water scarcity and high temperatures affect the synthesis of phenolic compounds. Prediction of the most important water stress parameters for distinguishing several sites in this study permitted a hierarchy of the five vineyards to be established. Analysis by CART has some advantages over other methods of classification or prediction for evaluating data from a pool of measurements of multiple vineyards. The first advantage is that this method

is nonparametric and thus does not require any assumptions regarding the distribution of the predictors, the response or the relationship between them, and their possible interactions. Another reason for the growing popularity of this technique is its interpretability. In general, the intuitive nature of decision trees makes them simpler to interpret relative to other methods of multivariate regression. The methodology presented here can be robustly applied to large datasets to detect patterns without making any assumptions about the distribution or variance of the data. The information from these types of studies can also be useful for making better management decisions for viticulture systems. A key advantage of the tree structure is its applicability to a wide variety of variables. In the particular case of the Priorat wine growing area, due to the complex orography, the CART technique is useful to segment several varied groups of plant and grape composition data, from very hetereogeneous vineyards. This study indicates the CART technique can be used to interpret larger data sets from different crops and other areas to help interpret the physiological results obtained.

5 | MATERIALS AND METHODS

5.1 | Site location and plant material

The study was performed at five sites: two sites (Site 1 and Site 2) located in an early mesoclimate (El Molar) and three sites (Site 3, Site 4, and Site 5) in a late-ripening mesoclimate (Porrera) at different altitudes. Sites of the early region El Molar (EM) were located at: Site 1 (41°9′90″N; 0°42′75″E, elevation 100m) and Site 2 (41°9′40″N; 0°42′38″E, elevation 200m). The following three sites were selected for the late region in Porrera (PO): Site 3 (41°10′51″N; 0°52′25″E, elevation 410 m), 450 m: Site 4 (41°10′50″N, 0°52′29″E elevation 450m), and Site 5 (41°10′57″N, 0°52′32″E elevation 490 m). Carignan old bush vines were studied (50–60 years) with an average load of eight buds per vine and were planted in a density of 5000–6000 vines ha^{-1}. Vines were planted in steep terraces with a slope of 15%–25%. The soils were composed of slate conferring a stony, dry, and poor soil. Furthermore, the soils were well-drained, as they contained a high proportion (between 70% and 90%) of large particles more than 2 mm in diameter.

5.2 | Climatic characterization during vintage in both regions

Weather stations (DECAGONmodel) located in each vineyard recorded various climate data, including temperature (°C), humidity (%), rainfall (mm), and radiation (W/m^2). VPD (vapor pressure deficit) was also calculated. The early region is located near the Ebro river, is characterized by higher temperatures in summer, and lacks cool breezes. In contrast, the late region experiences sea breezes that delay maturation. Vineyards located on hillsides and terraces are drier; however, the effect of the sea breeze (i.e., *garbinada*) decreases summer temperatures, increases the relative humidity, and decreases evaporation, resulting in delayed ripening. However, the cold, dry wind that blows from the northwest along the Ebro basin (i.e., *serè*) also affects the wine growing area of Priorat. The climate of the DOCa (Denominación de Origen Calificada) is characterized by cold temperatures during the winter and hot temperatures during the summer. The annual precipitation is between 450 and 500 mm, and rains are abundant between the end of October and November.

Data that characterize climatic variation between small plots are essential for improving crop management under such extreme conditions. The weather station (Agro-climatic network in Catalonia, XAC) provided supplemental data on the weather conditions in the study area. The climate in the Priorat region (Tarragona, Spain) is characterized by high temperatures during the summer, drought, and steep poor stony soils and is thus highly vulnerable to climate change. In the early mesoclimate (El Molar, EM), the minimum temperature differences between Site 1 and Site 2 were 7°C, except in early March to mid-May and the first 3 weeks of July, where the minimum temperature differences were up to 3°C lower in Site 1. These differences, along with a slightly higher maximum temperature in Site 2, resulted in a higher thermal amplitude (AT) on the vineyard, especially from mid-May to early July and from veraison (V) to ripeness (RP) (August 15–September 21). Approximately 40% and 42% of the total precipitation, in EM (El Molar, early ripening site) and PO (Porrera, late-ripening site), respectively fell in April, and the levels of precipitation were low during the summer months. Only moderate rain values were recorded in June (20 and 19 mm in EM and PO, respectively), indicating that the summer was dry. The average temperature during the summer months was high, reaching 23.2°C in June, 25.5°C in July, and 25.8°C in August in EM and 21.4°C, 23.4°C, and 23.9°C in PO in June, July, and August, respectively.

5.3 | Phenology

The effect of climate on phenology resulted in greater variability in budbreak and veraison (V) dates depending on previous budbreak temperatures and those recorded in the spring. A temperate vintage budbreak was delayed by 8 days in Sites 3, 4, and 5 and by 11 days in Sites 1 and 2 when compared with a warm vintage. However, in a temperate vintage, the differences were less notable at the beginning of bud break (BB) and veraison (V) between the early and late regions (3 and 5 days, respectively). The high temperatures in spring resulted in an earlier fruit set in the late region, which matched the date of fruit set in the early region. Moreover, the extended summer at the end of the ripening period caused the harvest date to be 15 days prior to the normal harvest date in the region. Most earlier studies examining the effect of climate on phenology have detected reductions in the amount of time between phenological stages; however, most previous studies have been conducted in cool climate vineyards (Bock et al., 2011; Jorqueta-Fontena & Orrego-Verdugo, 2010). Date of

harvest varied by 15 days between regions in the warm year, 10 days in the temperate year, and only a week in the warm year with seasonal temperature variability. A delay in bud break did not result in a delay in harvest; warm years in the late region resulted in an earlier harvest date. These observations are associated with high temperatures occurring in late August and even September in the Priorat, which results in accelerated grape ripening.

5.4 | Yields and grape ripening

Berry ripening was carefully monitored, and chemical analyses of the resulting wines were evaluated. During harvest, weekly samples of 400 berries were randomly harvested and then analyzed. Sugars (Brix), ATT (g/L total tartaric acidity), and the pH of the grape juice were determined. After crushing the whole berries, extraction of phenolic compounds was performed following a modified version of the Glories method (Nadal et al., 2010) to determine total (ANT T) and extractable anthocyanins (ANT E); %EA (extractability of Anthocyanins), %SM (seed maturity), and TPI (total polyphenol index) were also measured. OIV methods (International Organisation of Vine and Wine) were used to analyze alcohol by volume (ABV), total tartaric acidity (ATT), pH, anthocyanins, DMACA (flavan-3-ol by derivatization with *p*-dimethylaminocinnamaldehyde), and total tannins in wine. ANOVA was performed using the general linear model procedure. The Tukey test was used for post hoc analysis (XLSTAT statistical package, EXCEL) between plots.

5.5 | LWP

The LWP in each phenological stage, PS (pea size), V (veraison), and RP (ripeness), were measured using a pressure chamber (207 bar/3000 PSI pressure) (Model 600 PMS Instruments, Oaklands Park, Wokingham, United Kingdom) according to the technique described by Scholander et al., (1965). Leaf water potentials are reference measures of vine water status that have enabled solid reference thresholds of vine water status to be established. To ensure consistent readings, predawn LWP (Ψ_{PLWP}) was measured one to two hours before sunrise at 8:00 (6:00 solar time), when grapevine water status is at a maximum (Carbonneau, 1998), and midday LWP (Ψ_{MLWP}) was measured at 2:30 (12:30 solar time). In addition, primary (PLA) and secondary leaf (SLA) areas were measured during the PS, V, RP, and PH (postharvest) stages.

5.6 | Sample leaf preparation for ABA determination

Several long and tedious methods have been developed for the extraction and determination of ABA in plant tissue; however, some studies have developed more rapid approaches for the determination of phytohormones in plant material other than vine leaves (Riov et al., 1990; Setha et al., 2005). However, the establishment of a rapid method for determining ABA in vine leaves (López-Carbonell & Jáuregui., 2005), along with measurements of LWP, could provide important information for the classification of the water status of the vineyards.

Healthy leaves having reached approximately two-thirds of their definitive size were sampled from five vines per block and were bagged using Ziploc bags covered with a metalized high-density polyethylene reflective film to avoid additional leaf heating. This approach prevents the degradation of phytohormones, such as ABA. Samples were stored at −20°C. The methodology of López-Carbonell et al., (2009) was used for the extraction of ABA in Carignan leaves. Extraction solvent (Solution 1) was prepared with acetone/water/acetic acid (80:19:1, v/v/v). The solvent temperature was kept at −20°C. Reconstitution solvent (Solution 2) was prepared with water/acetonitrile/acetic acid (90:10:0.05, v/v/v). This methodology was improved by carefully weighing 4–5 g of fresh weight from a pool of different leaf samples and lyophilizing samples in a Telstar LyoQuest freeze dryer with a condenser temperature of −55°C, followed by powdering with mortar and pestle. Dried samples were carefully weighed in a 1.5-ml Eppendorf tube. Next, 1 mg of ABA internal standard was added to each of the three replicates at the beginning of the extraction procedure. A volume of 1.2 ml of extraction solvent (Solution 1) with the 300 mg of sample inside the Eppendorf was extracted in triplicate, and temperatures remained cool while samples were manipulated. The Eppendorf mixture was vortexed and left overnight at −20°C, followed by centrifugation at 15,000 ***g*** for 10 min at 4°C. Supernatants were pooled, dried under a nitrogen stream (Stuart, SBH200D), and reconstituted in 445 µl of reconstitution solvent (Solution 2), followed by stirring, vortexing, and centrifugation (10,000 ***g***, 10 min). Samples were filtered through a 0.22-µm PTFE filter (Millex Syringe-driven Filter Unit). Next, 5 ml of each sample was injected into the LC–ESI–MS/MS system. Internal standards were used for the calibration of ABA. The calibration curves for ABA showed high linearity (R^2 = .9959). The regression equation for the relationship between area (EIC) and ABA concentration (mg/L) was ABA = 1×10^6Area − 138.14. ABA standards were prepared daily. High correlation coefficients ($r > .995$) were obtained for concentrations ranging from 0.019 to 0.272 mg/L.

5.7 | Berry sampling and winemaking

The evolution of grape ripeness and wine composition at the five sites was followed at each of the two municipalities during the early (EM) and late (PO) mesoclimate. A total of 400 grape berries were randomly sampled. The total sugar content was measured by a refractometer. The pH was measured after homogenization of the juice. Small-scale fermentations were performed for each site in triplicate. Grapes were randomly sampled, de-stemmed, crushed into stainless-steel wine vats, and fermented after 3 days of cool maceration to extract the color and following the fermentation of all sugars. Potassium metabisulfite was added to a final concentration

of 20 ppm to preserve the products of oxidation processes until bottling. The wine did not undergo malolactic fermentation. The composition of wine was determined at all five sites. Specifically, alcohol by volume (ABV), total acidity (TA), pH, total anthocyanins (Ribéreau-Gayon et al., 2000), tannins, and flavan-3-ol (DMACH method) were determined.

5.8 | HPLC analysis of anthocyanins

High-performance liquid chromatography (HPLC) was used to quantify the amount of anthocyanins and procyanidins in wines from the five treatments. Triplicates from each sample were analyzed. Anthocyanins were quantified using calibration curves of the most similar compound: malvidin-3-glucoside. Total amounts of anthocyanins were given in mg/g berry (grapes) and mg/L (wines). The different phenolic compounds analyzed were tentatively identified according to their order of elution and the retention times of pure standards (catechin, epicatechin, catechin gallate, epicatechin gallate, procyanidin B1 and B2) (Fluka). Procyanidin dimers in grape extracts were identified by analytical HPLC and comparison with authentic standards. The (–)-epicatechin *O*-gallate and B2-3′-*O*-gallate were collected from the HPLC column, and their structures were elucidated by NMR.

5.9 | Chromatographic conditions for anthocyanin analysis

Column Zorbax Eclipse Plus C18 150 × 2.1 mm, 3.5 μm (SFF-CXX, P/N 959763-902) and Precolumn Zorbax Eclipse Plus-C18 12.5 × 4.6 mm, 5 μm (SFF-C002, P/N 820950-936) were assembled over P/N 820888-901. *HPLC conditions*: injection volume 5 μl; mobile phase A Water HPLC-grade (0.2% trifluoroacetic acid); mobile phase B methanol (0.2% trifluoroacetic acid); column temperature 50°C; Detector DAD (diode array detector) (Peak width > 0.1 mm (2 s); storage of all 190–700 nm step 2 nm; slit 4 nm; margin for negative absorbance 100 mAu. *ITMS conditions*: ionization source ESI positive; ion trap analyzer (capillary 3,500 V, target mass 493 m/z, comp stability 100%, trap drive level 100%, scan 100–900 m/z, ICC smart target 500,000, max accu time 200 ms, average 5). The anthocyanidin monoglucosides of the skin extracts and wines were chromatographed by HPLC using a Beckman Ultra sphere (C18) ODS (250 × 4.6 mm i.d.) column, and detection was carried out at 520 nm. The solvents were A, H_2O/HCOOH (9:1), and B, CH_3CN/H_2O/ HCOOH (3:6:1). The gradient was 20%–85% B for 70 min, 85%–100% B for 5 min, and then isocratic for 10 min at a flow rate of 1 ml/min. The content in free anthocyanins was determined using a calibration curve (based on peak area), which was established using malvidin 3-glucoside. Standard solutions were subjected to the same procedure [concentration (mg/L) = 803.7 × (do − d) + 15.13].

The contents of free anthocyanins were determined using calibration curves (based on peak area), which were established using malvidin 3-glucoside. Standard solutions were subjected to the same procedure ($y = 0.7968x + 7.5756$, $R^2 = .9774$). The anthocyanidin-3-monoglucosides and respective acetylated and coumaroylated glycosides were identified based on their UV-Vis spectra and retention times. The anthocyanidins were identified by HPLC by comparison with internal standards. The calibration curves were obtained by injecting standards with different concentrations of malvidin 3-glucoside (Sigma). The range of linear calibration curves was from 0.1 to 1.0 mg/L for the lower concentration compounds ($R^2 > .996$), 0.1 to 5.0 mg/L for intermediate concentration compounds ($R^2 > .987$), and 10.0 to 200.0 mg/L for the higher concentration compounds ($R^2 > .987$). Unknown concentrations were determined from the regression equations, and the results were expressed in mg of malvidin 3-glucoside per berry. Repeatability of this method from extraction to HPLC analysis for four samples of the same batch of grape skins had a coefficient of variation <7%.

5.10 | Statistics

The water potential, leaf temperature, and grape and wine composition were evaluated through one-way ANOVA, and when $p < .05$, Tukey post hoc tests were used. A Pearson correlation matrix was calculated for all parameters with a significance level (α) of 0.05.

CART (classification and regression trees) analysis was performed using XLSTAT (Microsoft Excel statistical add-in). The decision tree method is a powerful and popular predictive machine-learning technique that is used for both classification and regression (Breiman et al., 1984). Thus, the methods are also known as Classification and Regression Trees (CART). The algorithm of decision tree models repeatedly partitions the data into multiple subspaces, so that the outcomes in each final subspace are as homogeneous as possible. Among all measured variables, the CART technique acts as a predictive model that shows the more significant variables to distinguish each final subspace. The tree models predict the outcome by asking a set of *if–else* questions. Regression tree analysis predicted the outcome as a real number (leaf temperature and water potential). The start of the tree was at the root node; for each variable, CART finds the set that minimizes the sum of the node impurities in the two child nodes and chooses the split that gives the minimum overall variable and set. The measure of the node impurity is based on the distribution of the observed values in the node; splitting stops if the relative decrease in impurity is below a pre-specified threshold.

ACKNOWLEDGMENTS

We would like to aknowledge the University Rovira i Virgili, Biochemistry Department, Viticulture Research Group. Partial support for this work has been received from the Agency for Management of University and Research Grants (AGAUR) of the Government of Catalonia (grant 2017 SGR 705). Also we would like to acknowledge the anonymous reviewers for helping to improve the manuscript and supporting information.

AUTHOR CONTRIBUTIONS

J.M.M designed the statistical data analysis A.S performed the experiments. M.N supervised the water stress experiments, and M.L provided technical vineyard data assistance A.S. agreed to serve as the author responsible for content and communication.

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How to cite this article: Sánchez-Ortiz A, Mateo-Sanz JM, Nadal M, Lampreave M. Water stress assessment on grapevines by using classification and regression trees. *Plant Direct*. 2021;5:e00319. https://doi.org/10.1002/pld3.319