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Phenology and grape ripening characteristics of cv Tempranillo within the Ribera del Duero designation of origin (Spain): Influence of soil and plot characteristics

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ABSTRACT

The aim of this research was to evaluate the variability of phenology and ripening characteristics of the Tempranillo variety within the Ribera del Duero Designation of Origin (Spain). This area covers approximately 115 km along the Duero River, where Tempranillo is the main variety cultivated. The analysis included the information recorded during the period 2004–2013 in 20 plots for phenology dates and 26 plots for grape characteristics. The variability in soil, phenology, grape quality and plot characteristics throughout the Ribera del Duero DO as well as their relationships were evaluated using multivariate analysis. Four different groups of plots were characterized as distinct from each other, with differences in elevation, distance to the Duero River and soil type. The differences in phenology among groups started during flowering and were observed through the end of the growth cycle. Despite the high phenological variability driven by year to year variations in climate characteristics, it was possible to define the soil and plot characteristics that favor advanced phenology within the Ribera del Duero DO. Regarding grape ripening characteristics, the highest acidity and anthocyanin concentrations were found in plots with soils with higher clay and organic matter content. The effect was greater in the wet and intermediate years, than in dry years. High variability in phenology and ripening characteristics is found within the Ribera del Duero related to site soil and landscape characteristics, and from year to year due to climatic conditions. Zones with common characteristics and similar response have been identified within the area. The results highlight the potential of establishing viticultural zones with differences in vineyard treatment and management and the elaboration of site specific wine styles from those zones.

1. Introduction

The history of viticulture activity in the Ribera del Duero (Spain) is strongly tied to the landscape and climate of the region, along with significant cultural and social influences in wine production. Vineyards in this area date back to the Roman time, with significant fluctuations in production throughout the centuries. Early on viticultural activity reached a consolidation with a stable production in the 10th and 11th centuries. During the following centuries vineyards and wine became important in the economic and cultural development of the Ribera del Duero, and increasing the production and distribution to other Spanish areas.

The present Ribera del Duero Designation of Origin (DO) was established in 1982 and since then the surface area planted has increased from 6460 ha of vineyards officially registered in 1985 to approximately 21,500 ha in 2013, and the region has become world renowned for being one of the highest quality red wine producing regions. This area accounts for 2.7% of the vineyard area covered by DO registration in Spain (MAGRAMA, 2014); however the quality of its wines has reached the top of the international wine market being recognized worldwide. Today the total grape production stands at around 90 million kg, with an average yield that approaches nearly 4500 kg/ha (www.riberadelduero.es). Tempranillo is the dominant variety planted in the DO, accounting for over 95% of the surface area with more than 20,500 ha and a production that was just over 83 million kg in 2012 (www.riberadelduero.es).

Tempranillo is a thick-skinned variety with a high anthocyanin concentration that makes for deep-colored wines with moderate tannins and moderate acidity. The variety provides the structure of

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some of the finest red wines from Spain and Portugal, and today has spread to other regions such as Argentina, Australia, California and other viticultural regions around the world where the terroir is suitable. Spain is the world's largest Tempranillo producer (Anderson, 2013) with the Ribera del Duero DO as one of the main contributors to the countries production. The proportion of Tempranillo grapes in the red wines of the DO is regulated to exceed more than 85% (www.riberadelduero.es, Regulation of DO), however many of the wines are produced with 100% Tempranillo. The Ribera del Duero DO has also been recently named as the world's wine region of the year in 2012 (Wine Enthusiast Annual Wine Star Awards 2012) (www.drinkriberawine.com/2012/11/wine-region-of-the-year/).

Tempranillo typically exhibits a late budburst due to the cold winters and springs in the growing areas, but ripens early in areas where there is relatively large day–night temperature differences in the summer and early fall. It grows in topographically diverse regions that have high diurnal temperature variations which in turn help them to retain their natural acid balance. Vineyards in the Ribera del Duero area extend generally east-west about 115 km along the Duero River (Fig. 1). However, within the region vineyards are planted over relatively large differences in elevation and soil characteristics, which combine to contribute to variations in vineyard management, fruit production, and wine style differences. While vineyards in the region have historically been concentrated at higher elevations (up to 900 m) and often on hillside slopes, newer sites have been developed at lower elevations (between 750 and 800 m) along the river that contain more fertile soils. The differences in elevation of the vineyard sites tend to affect grapevine development and grape quality as well as the productive potential. These differences create a need to pay greater attention to spatial variations in vine management owing mainly to microclimatic aspects affecting the health and maturation of the grapes.

At present, vineyards have spread to places with different conditions including drier landscapes and on soils that are not very fertile. As such, adaptation of farming operations to variations in soil and climate are required. Thus, approximately 15% of the total vineyard surface area includes the use of irrigation to mitigate the effects of water stress. Tempranillo has also been traditionally cultivated as bush vines or in a "goblet" form, which allows the vines better development and the production of fruitier flavors. However, today there are numerous variations in training such as vertical shoot positioning that attempt to help manage the variations in soil and microclimates. Also, various cultivation methods have been adopted that help control the grape yield (since the DO establishes a maximum of 7000 kg/ha), mostly through cluster thinning and optimizing the vine microclimate through vegetation management. These operations include shoot thinning (or green pruning), topping, and lateral shoot and leaf removal (Yuste, 2008).

Soil characteristics together with climate and topography influence grapevine growth and fruit qualities and constitute one the elements of the "terroir effect" (Reynard et al., 2011; van Leeuwen et al., 2004). Climate arguably has the greatest influence on the suitability of the environment for grapevine growth and quality wine production (Hidalgo, 1999). The effect of climate, and in particular the effect of temperature on grape growth and composition, has been widely evaluated in different wine- producing regions worldwide and considered critical to characterise both wines and wine-producing regions (Bindi et al., 1996; Jones et al., 2005; Hall and Jones 2010; Szenteleki et al., 2011; Xu et al., 2012; Back et al., 2013; Webb et al., 2013; Bonada et al., 2015, among others). Climate affects almost all variables of grape composition, as well as the speed by which grapes ripen. High temperatures affect not only sugar development (Coulter et al., 2008) and acid respiration (Lakso and Kliewer, 1975; Cartechini and Palliotti, 1995; Sweetman et al., 2014) but also components that are important for color and aroma characteristics. Thus, temperature plays and important role on flavonoid development (Huglin and Schneider, 1998), anthocyanin concentrations (Coombe, 1987; Tarara et al., 2008), proanthocyanidin (Cohen et al., 2012 Zamora, 2003) and on various aroma compounds (Duchêne and Schneider, 2005; Reynolds and Wardle, 1993). Other climatic variables such solar radiation or water distribution are also important for the optimum development of color and aroma during ripening (Sebastian et al., 2015; Gregan et al., 2012), and also affect berry sizes and overall yield (Ubalde et al., 2010). However, within a specific climate zone, soil is the most important environmental factor controlling within vineyard vine development and fruit or wine quality (Sotés and Gómez-Miguel, 2003). Previous studies have demonstrated the relationship between some soil properties and grape and wine characteristics. In particular, soil physical properties essentially govern the potential volume of soil that can be explored by roots. Soil particle distribution and the associated pores between them affects, both directly and indirectly, many physical, chemical and biological aspects of the soil including soil strength, water and nutrient movement, soil aeration, soil hydraulic properties and drainage conditions (Lanyon et al., 2004; Seguin, 1986; Costantini et al., 2006). Relatively deep, well-drained soils are required for limiting waterlogged vine roots ultimately forcing deep root penetration to find water during times of seasonal drought stress. Poor soils usually promote smaller yields of grapes with more concentrated flavors, while fertile soils lead to overgrown vines which has an important impact on berry quality (optimum berry quality is seldom achieved if vines are excessively vigorous).

Although the lack of water is mainly associated with climate, storage of water in soil and root access to the stored water is dependent on soil physical properties. Water availability determines the vine water status (Costantini et al., 2010), which often plays a major role in determining the sensory characteristics of wines. Vine water status has an important impact on the phenolic composition of berries and seeds (Deloire et al., 2005), particularly during certain stages of their growth (Esteban et al., 2001; Sipiora and Granda, 1998). In addition, soil pH and organic matter content, have been highlighted among those factors with strong influences on phenolics (Gómez-Míguez et al., 2007).

Grape-growing is not very demanding in terms of soil chemistry and conditions. However, extremely acidic or alkaline soils hinder grape development, so soil pH affects the varietal selection to be planted in a particular vineyard. Furthermore, soil type and soil mineralogy influence vine nutrition and ultimately the final wine characteristics (Lambert et al., 2008). Although the relationship between soil minerals and wine quality are difficult to categorize precisely, they are usually established when severe deficiencies affecting vine growth occur (van Leeuwen et al., 2004). Some studies have shown an effect of soil cations on grape composition, which can influence wine quality (Mackenzie and Christy, 2005). Soil salinity can also affect vine development, however the response of grapevines to salinity has been shown to vary depending on the grape cultivar and rootstock used. Therefore, there has not been a universal relationship established to describe the effect of salinity on vine growth, yield and grape composition (Lanyon et al., 2004).

Given the important ties between site characteristics and vine growth and fruit quality along with the expansion of vineyards into new areas in the Ribera del Duero region, the main purpose of this research was to investigate the spatial variability of grapevine phenology and grape quality aspects as related to soil and plot characteristics. Phenology and ripening parameters were observed during the period 2004–2013, using 20 plots for phenology, and during the period 2003–2013, using 26 plots for ripening distributed throughout the Ribera del Duero DO. The influence of soil and plot characteristics on vine development was evaluated

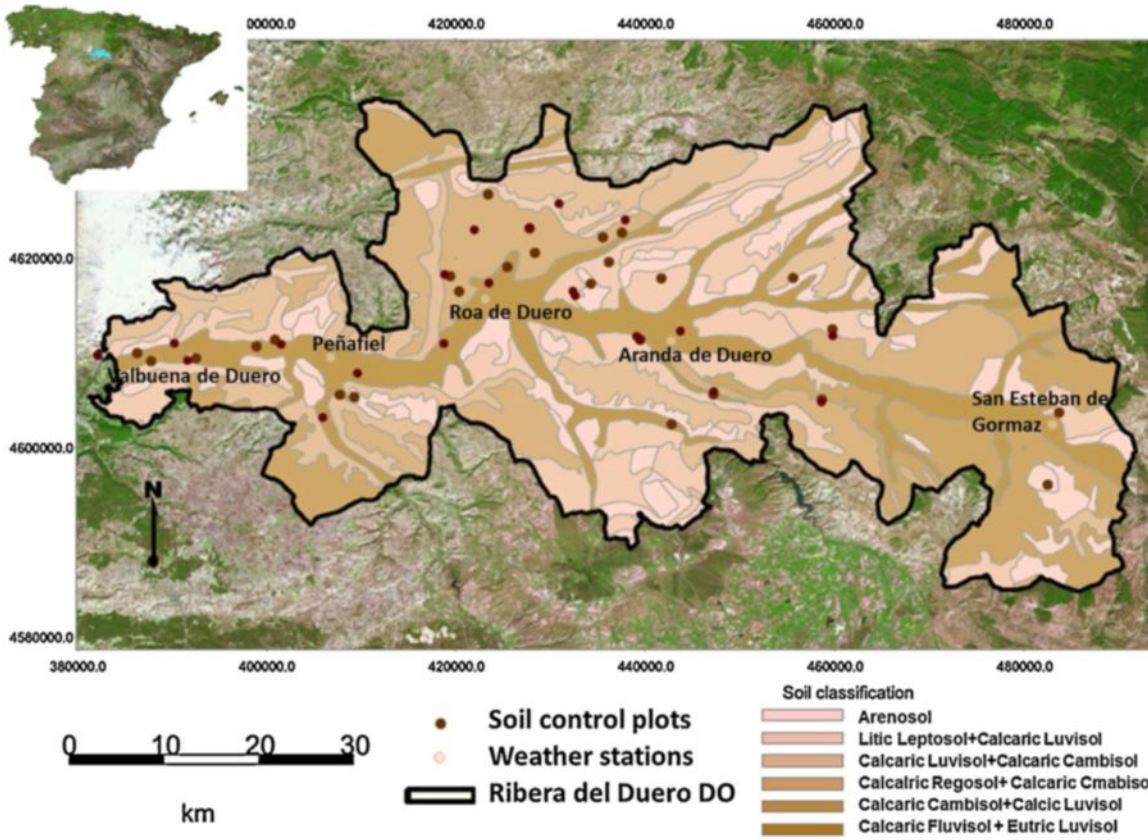

Fig. 1. Location of the weather stations and, the plots used for soil analysis and main soil types in the study area.

using multivariate statistical techniques (cluster analysis and principal component analysis).

2. Study area

The Ribera del Duero Designation of Origen (DO) is located in the northern plateau of the Iberian Peninsula. Vineyards of the Ribera del Duero DO extend about 115 km along the Duero River, from Quintanilla de Onésimo (Valladolid) to San Esteban de Gormaz (Soria) (Fig. 1), with elevations that range between 720 to slightly more than 1000 m a.s.l.

2.1. Climate characteristics

The climate is temperate with dry winters and hot summers in the western part and temperate with dry winters and temperate summers in the eastern part (AEMET, 2011). The mean annual temperature ranges between 10.2 and 12.0 °C, with mean maximum temperatures around 18.4 °C and mean minimum temperatures ranging between 4.5 and 5.0 °C. Significant differences in the growing season maximum temperatures (TGSmax) and in the maximum extreme temperatures exist between both extremes of the area with the lowest temperatures recorded in the eastern part of the Ribera del Duero area. Significantly lower minimum temperatures also exist in the eastern part of the area (Ramos et al., 2015). The mean annual precipitation ranges between 413 and 519 mm with the main rainfall periods during April–May and October–November–December. Climate data for the period analyzed (2003–2013) were recorded at six meteorological stations distributed along the Ribera del Duero area (SD: Sardón de Duero; VD: Valvuena de Duero; P: Peñafiel; RD: Roa de Duero; AD: Aranda de Duero; SEG: San Esteban de Gormaz) (Fig. 1), and belong to the Agencia Estatal de Meteorología (AEMET, Spain). Daily maximum and minimum temperature and precipitation were recorded at each station. From this information, the average temperature and precipitation corresponding to the growing season and that recorded during the hydrological year (Oct–Sep) and in each phenological period were calculated. Additional indexes such as the number of frost days (ndT0), the number of days with $T > 30$ °C, and important viticultural bioclimatic indexes (growing degree-days) were calculated for each year and station.

2.2. Soil characteristics

From a geological point of view, the Ribera del Duero is part of the large septentrional plateau formed by a basement filled with Tertiary deposits. Most of these deposits consist of layers of ochre and red loamy and clayey sands. The mid and low terraces from the Duero River consist of Quaternary alluvial deposits. According to the WRB (2006) classification the main soil types in the area are *Calcaric Cambisols, Eutric Cambisols, Calcic Luvisols, Calcaric Fluvisols, Eutric Fluvisols* and in less proportion *Lithic Leptosols* and *Calcaric Regosols* (Table 1).

In this study, 40 plots spatially distributed throughout the Ribera del Duero DO area (Fig. 1) were selected. Most plots were located along the Duero river terraces and in the central part of the area, where soils are ochre and red sands and clays from the Tertiary era. All plots were located in areas suitable for vine cultivation according to the classification done by Gómez-Miguel (2003). Soil characteristics of the study plots were obtained from the Castilla y León Soil map (IRNASA_400k) and completed with soil properties for each study plot for which phenology and grape quality data were obtained. Soil samples were taken from the surface (0–20 cm) in each plot at different points to prepare a composite sample. Three composite samples were analyzed for each plot. Each sample was homogenized, air dried and sieved through a 2 mm mesh. Soil parti-

Table 1
Main soil types and averages of various soil surface characteristics found in the studied area.

Soil type association	Number of plots	Clay (%)	Silt (%)	Sand (%)	Organic Matter (%)	pH
Calcaric Regosol + Calcalric Cambisol	6	22.6 ± 5.8	38.18 ± 3.0	39.3 ± 4.7	1.85 ± 0.70	8.6 ± 0.1
Calcaric Cambisol + Calcic Luvisol	18	22.48 ± 7.7	40.1 ± 11.9	37.8 ± 16.1	2.10 ± 0.80	8.3 ± 0.3
Calcaric Fluvisol	14	19.3 ± 9.6	27.9 ± 12.5	52.8 ± 18.5	2.14 ± 0.84	8.3 ± 0.3
Cambic arenosol	1	21.9	41.9	36.7	2.15	8.3
Lithic Leptosol	5	23.8 ± 6.2	36.2 ± 12.2	40.0 ± 9.2	1.90 ± 0.97	8.2 ± 0.2

cle distribution, organic matter content, pH, electrical conductivity (1:5 water extract), structure and permeability of the soil surface of each plot were considered in this study. Measures were performed following the methods proposed by the Soil Survey Staff (2011). In addition, the coarse element fraction was also evaluated.

2.3. Phenology and grape quality

Vine information was provided by the Consejo Regulador of the Ribera del Duero DO. All plots analyzed were planted with the Tempranillo variety and covered different landscapes, vine age and training systems used but are very representative of the vineyards in the region. The plant material of the vines planted in the observed plots comes from homogeneous populations of the Tempranillo variety, but comes from different clones. The use of individualized clones in the newer vineyards planted in the Ribera del Duero is a more recent trend than the vineyards included in this research. All the vineyards studied are more than 10 years old and considered mature plantings in full productive capacity. They cover a diversity of vine ages between 11 and 70 years, with an average of 26 years (Table 2) and correspond well with the range of vineyard ages in the entire study area.

2.3.1. Phenology data

The analysis of spatial and temporal variability in phenology was based on the information recorded in 20 plots distributed throughout the Ribera del Duero area (Fig. 2) for the period 2004–2013. Phenological dates (Baggiolini classification) corresponding to C (bud break), G, I (bloom), K, L and M (veraison) stages plus harvesting date were averaged over each plot and analyzed. The data given for each stage in each plot corresponded to that at which more than 50% of vines had reached the phenological stage. Average information about harvest beginning and ending dates for the whole area referred to the period 1980–2013 was also examined.

2.3.2. Grape quality data

The analysis of ripening quality parameters was based on 26 plots distributed throughout the Ribera del Duero (Fig. 2). Parameters including pH, titratable acidity, malic acid, total soluble solids, total and extractable anthocyanins, color intensity and berry weights recorded from 2003 to 2013 were evaluated. In each plot 100 berries were randomly sampled from the central and lower part of the clusters, according to the criteria proposed by Jordan and Crosser (1983) and weighed for the analysis. In addition, a sample of 200 berries were crushed and centrifuged for further analysis of fruit composition. The crushed berry samples were carried out in duplicate and pH was measured using a pH electrode; total acidity was measured by titration with NaOH 0,1N; malic acid was evaluated enzymatically by measuring the L-malato concentration by absorption at 340 nm; the total soluble solids were measured by refractometry and expressed in °Beaumé; total and extractable anthocyanins were measured according to the Saint-Cricq method in extracts at pH 3.2 and pH 1, respectively (absorbance at 520 nm); and the color index was obtained from the absorbance at 420, 520 and 620 nm.

2.4. Data analysis and methods

2.4.1. Spatial variability of phenology

In order to characterize the spatial variability of phenology in the region, the plots were classified using a hierarchical cluster analysis taking into account the dates of the different phenological stages (C, G, I, L and M) across all plots.

Among the potential different criteria for establishing clusters, Ward's minimum variance method was used as it has been pointed out as a very efficient criterion (Milligan 1980; Gong and Richman 1995). Ward's method calculates the distance between two clusters as the sum of squares between the two clusters added up over all the observations. This method attempts to minimize the Sum of Squares (SS) of any two (hypothetical) clusters that can be formed at each step. At each generation, the within-cluster sum of squares is minimized over all partitions obtainable by merging two clusters from the previous generation. Variables were standardized, i.e., transformed to variables with a mean = 0 and variance = 1. The number of clusters to be retained was defined by taking into account the agglomeration distance measuring the inter-cluster continuity and the clustering coefficient. The cut-off point was established when the distance between one step and the next one was greater than twice the average distance. The average values of each cluster were evaluated. The variability in phenology dates was related to soil characteristics, elevation, slope, aspect, vine age, irrigation/non irrigation and training system in each of the locations.

2.4.2. Spatial variability of grape quality

The relationship between grape quality parameters and soil properties and their spatial variability was analyzed by principal component analysis using the grape quality variables. Principal component analysis (PCA) is widely applied in atmospheric sciences and climate research to help identify the underlying pattern or modes of variability in complex, interrelated data. PCA has been applied in previous studies to characterize the relationship between soil characteristics and grape quality (Priori et al., 2013). The varimax rotation criterion was applied in order to improve the orthogonality of the components. The first few components were retained, which represented the majority of the variability in the data matrix.

The plots were then classified into groups based on the variables grouped in the retained factors. Due to the high variability observed in the grape quality values recorded over the different years of the period studied, the years analyzed were classified into three groups according to water availability in each phenological stage in each year. The three groups were classed into wet years, in which water restriction was small; dry years with high water deficits in all phenological stages; and years related to intermediate conditions. The years included in the three groups were respectively: 2007–2008–2010; 2005–2009–2011–2012–2013; and 2003–2004–2006. The establishment of these groups of years was done in previous work in the region (Ramos et al., 2014). Soil properties were used as auxiliary variables to interpret the plot classification. The plots were then classified in a cluster analysis (with similar criteria as described previously) based on the year classification (wet, dry or interme-

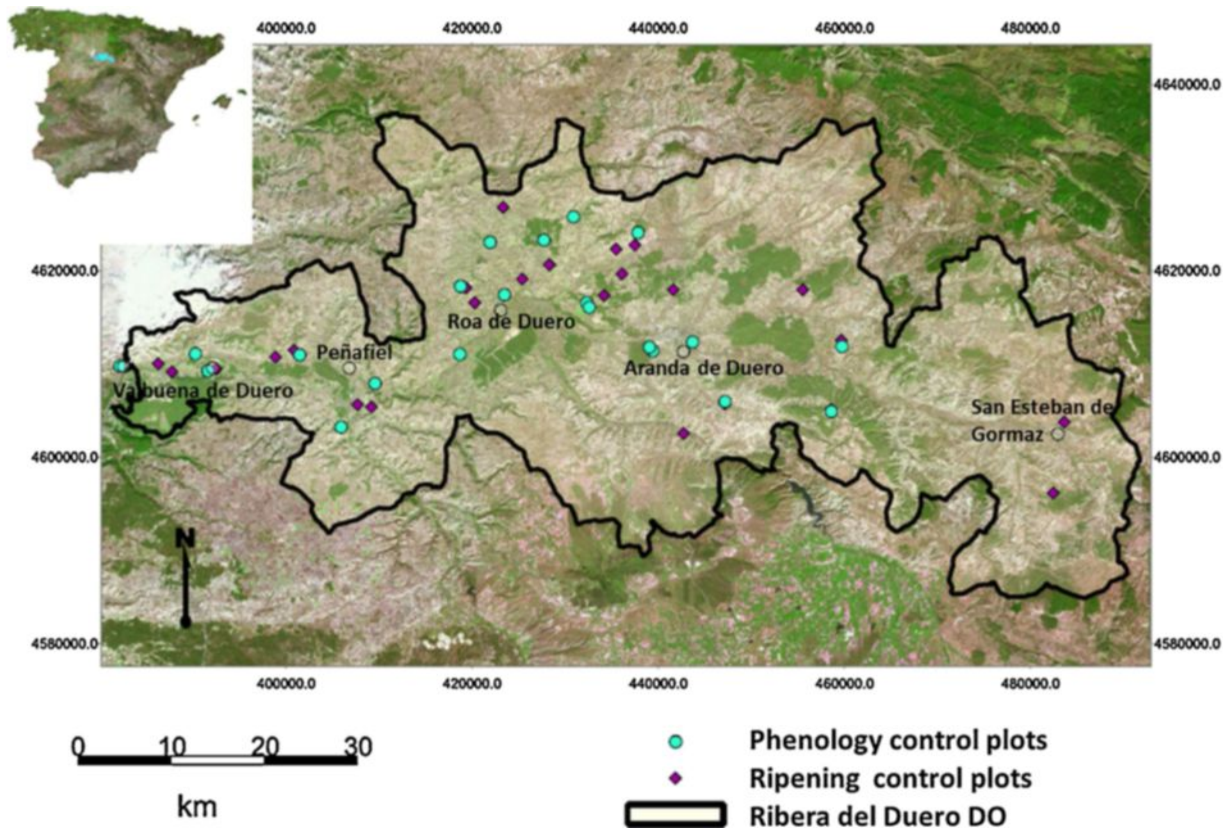

Fig. 2. Location of plots used in this study for phenology and ripening control analysis.

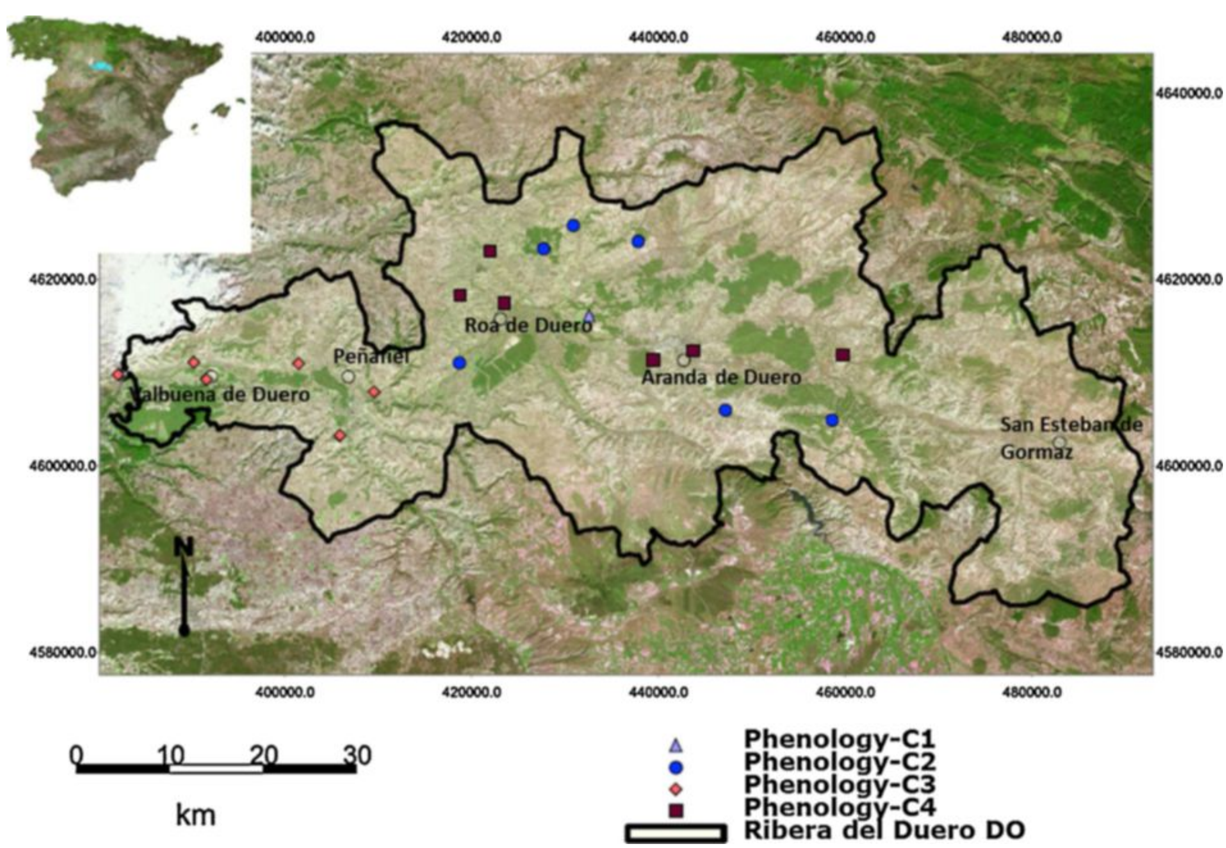

Fig. 3. Plot classification according to phenological dates recorded from 2004 to 2013. C1 through C4 represent the clustering of the plots according to the timing of the main phenological events during the growing season.

Table 2
Plot characteristics used in the phenology analysis.

Municipality	ID	Soil classification	Slope (%)	Elevation a.s.l. (m)	Distance to river (m)	Age (years)	Training System	Irrigation (Yes/No)
Gumiel de Mercado	P2	*Calcaric Cambisol + Calcic Luvisol*	3.0	803	1808	12	VT	N
Valbuena de Duero- Quintanilla	P7	*Calcaric Fluvisol*	4.0	735	1391	52	VT	N
Castillejo de Robledo	P9	*Calcaric Cambisol*	3.0	985	1633	17	VT	N
Peñaranda de Duero	P11	*Calcaric Regosol + Calcaric Cambisol*	13.8	878	2807	64	VT	N
Aranda de Duero	P13	*Calcaric Fluvisol*	3.6	798	527	15	VT	Y
Sotillo de la Ribera	P14	*Calcaric Regosol + Lihtic Leptosol*	7.0	875	9616	49	VT	N
Roa	P15	*Calcaric Fluvisol*	2.7	784	735	11	VT	Y
Aranda de Duero	P17	*Calcaric Cambisol + Calcic Luvisol*	2.9	834	1528	17	VT	N
Olivares de Duero	P19	*Calcaric Cambisol*	3.5	751	858	15	VT	N
Pesquera de Duero	P23	*Calcaric Fluvisol*	2.6	771	1541	19	VT	N
Curiel de Duero	P25	*Calcaric Fluvisol*	4.5	754	405	16	VT	N
Fuentelcésped	P26	*Calcaric Cambisol*	1.5	851	5041	38	VT	N
Pedrosa de Duero	P27	*Calcaric Cambisol + Calcic Luvisol*	3.6	817	4711	32	VT & G	N
Quintana del Pidio	P29	*Calcaric Cambisol + Calcic Luvisol*	8.4	865	11343	70/10	VT	N
Anguix	P30	*Calcaric Cambisol + Calcic Luvisol*	3.9	835	6554	24	VT & G	Y
La Horra	P31	*Calcaric Cambisol + Calcic Luvisol*	3.7	828	5995	26	VT	N
San Martín de Rubiales	P32	*Calcaric Fluvisol*	3.8	803	259	11	VT	Y
Valbuena de Duero	P33	*Lithic Leptosol*	6.8	749	425	13	VT	Y
Peñafiel-Aldeayuso	P34	*Calcaric Fluvisol*	3.0	757	731	14	VT	N

VT: vertical trellis; G: Goblet.

diate) and according to two groups of grape quality parameters: acidity and anthocyanins.

3. Results

3.1. Plot and soil characteristics

Table 1 summarises the main soil types found in the studied plots and the average values for some of the soil characteristics (soil particle distribution, organic matter and pH). Texture ranged between sandy clay loam to loam, with clay contents ranging between 4.7 and 47.0%; silt content ranged between 2.5 and 69.0% and sand content ranged between 10.0 and 77.9%; and with permeabilities that ranged between very low (<0.15 cm/h) and moderate (2–6 cm/h). The pH values ranged between 8.0 and 8.8. The organic matter content varied between very low values (<0.5%) and values higher than 4.0%, and the electrical conductivity had values between 0.09 and 0.25 dSm^{-1}.

Table 2 shows numerous characteristics (location, elevation, distance to the river, slope, training system and irrigation) of the plots used in the phenology analysis. The plots were distributed throughout the DO, covering elevations between 749 and 985 m a.s.l., moderate slopes that average roughly 5 percent, and that have distances to the Duero River between 259 and 11,343 m. Most plots have vertical trellis training system and are not irrigated, and the age of the vines ranged between 10 and 70 years.

3.2. Climatic characteristics during the period of study

The average climatic characteristics of for each station for years included in this study (2004–2013) are summarized in Table 3. The mean maximum (TmaxGS) and minimum temperature (TminGS) during the growing cycle were 25.1 and 9 °C, respectively, with an average number of frost days (ndT0) of 77.4 and with maximum temperatures >30 °C of 47.4 days. The number of frost days recorded in the period analyzed were lower than the average for the period 1980–2012 in the eastern part of the area, while in the western part they were higher than the average. Regarding the number hot extremes (ndT30), the results were the opposite. Differences in the WI and HI growing degree-day indices between both extremes of the area were also found (WI ranged between 1190 and 1578 and HI ranged between 1972 and 2328). Precipitation during the growing period (PGS) was 180.0 mm, on average, mainly recorded before veraison, with significantly higher values in the extreme western portion of the Ribera del Duero region. PGS represented about 45% of annual precipitation.

Within the growing period, more than 50% of the precipitation took place during the bud break-bloom period, while during the bloom–veraison and veraison–harvest periods precipitation is relatively low (about 20–24% of growing season precipitation in each period). Low late season precipitation produced water deficits during the last stages of the growth cycle, which have significant influences in the non-irrigated vineyards.

However, during the period analyzed significant differences were found among years. The average values of temperature and

Table 3
Mean values andstandard deviation (m ± std) of the climatic characteristics recorded at 6 weather stations distributed along the Ribera del Duero area during the period 2004–2013 (SD: Sardón de Duero; VD: Valvuena de Duero; P: Peñafiel; RD: Roa de Duero; AD: Aranda de Duero; SEG: San Esteban de Gormaz) (TmaxGS (maximum temperature during the growing period), TminGS (minimum temperature during the growing period), ndT0 (number of frost days), ndT30 (number of days > 30 °C), PGS (precipitation during the growing period); PHY (precipitation during the hydrological year), PBB (precipitation during the budburst to bloom period), PBV (precipitation during the bloom to veraison period), PVH (precipitation during the veraison to harvest period), ETcGS (crop evapotranspiration during the growing period), WI (Winkler index), HI (Huglin index).

Station	TMaxGS (°C)	TMinGS (°C)	ndT0 (days)	ndT30 (days)	PGS (mm)	PHY (mm)	PBB (mm)	PBV (mm)	PVH (mm)	ETcGS (mm)	WI (gdd)	HI (gdd)
SD	25.7 ± 1.3	9.0 ± 0.7	51.9 ± 14.9	53.4 ± 13.5	172.4 ± 98.0	364.8 ± 136.0	108.8 ± 65.4	32.4 ± 27.8	31.2 ± 37.0	582.9 ± 21.9	1579 ± 210	2328 ± 195
VD	25.7 ± 1.0	9.3 ± 0.9	83.8 ± 12.9	50.6 ± 14.1	158.7 ± 70.2	394.6 ± 101.2	83.8 ± 52.2	35.7 ± 26.5	39.1 ± 25.7	591.6 ± 22.2	1397 ± 197	2180 ± 188
P	24.9 ± 1.2	9.3 ± 0.7	83.4 ± 12.7	41.5 ± 11.7	144.6 ± 49.8	347.2 ± 94.4	86.9 ± 40.3	32.5 ± 12.7	25.2 ± 14.5	574.6 ± 30.5	1350 ± 166	2104 ± 181
AD	24.7 ± 1.9	8.5 ± 0.5	66.8 ± 18.2	52.9 ± 14.2	183.4 ± 50.8	427.6 ± 106.2	109.8 ± 41.8	37.6 ± 16.2	36.0 ± 20.1	584.9 ± 77.2	1271 ± 228	2080 ± 283
RD	24.9 ± 1.0	8.9 ± 2.1	91.5 ± 23.9	43.5 ± 13.1	191.7 ± 57.9	429.1 ± 121.1	113.0 ± 45.0	43.5 ± 17.0	35.1 ± 21.1	591.8 ± 143.4	1191 ± 378	1973 ± 482
SEG	24.7 ± 1.2	9.1 ± 1.4	87.6 ± 16.2	42.5 ± 11.2	229.3 ± 112.0	480.2 ± 156.6	142.2 ± 73.3	47.6 ± 35.3	42.6 ± 35.6	568.4 ± 35.2	1279 ± 164	2055 ± 194

precipitation variables for each year of the period analyzed are shown in Table 4. Very hot years such as 2005, 2009 and 2011 and years with low temperatures, such as 2004, 2007, 2008 and 2013 were recorded. In addition, very wet years such as 2007 and 2008 with high precipitation (both annual and during the growing season), and very dry years, such as 2005 and 2009 were recorded. The period also included years with low precipitation during the growing season but high precipitation accumulated during the year (such as 2004 and 2011), which meant a significant reserve for the crop cycle. Thus, the period analyzed included different climatic conditions, which can condition the vine response for a given location, as a function of the interaction between soil characteristics and the climate of the year (Acevedo-Opazo et al., 2008).

3.3. *Phenology variability*

Table 5 shows the average dates and their standard deviation for different stages (according to the Baggiolini scale) for the entire data set analyzed (2004–2013). On average, budburst took place on April 27th; bloom on June 15th and veraison on August 15th. However there were differences between plots and between years. The variability within plots was attributed to different site factors, which are discussed below.

The cluster analysis, taking into account the dates referring to stages C, G, I, L and M from all plots, produced a four group classification (Fig. 3). Plot and soil characteristics (elevation, slope, aspect, vine age, irrigation/non irrigation and training system, textures and organic matter content) were used as auxiliary variables and allowed the interpretation of the groups that were retained. Three clusters grouped a similar number of plots (six plots in each cluster) while the fourth group (cluster 1; C1 in Fig. 3) had only one plot (p2) that was linked at high distance to cluster 2 (C2 in Fig. 3).

The average characteristics of the plots included in each cluster are indicated in Table 6. The main differences among clusters 1 and 2 were the elevation, the distance to river and the soil characteristics. In plot 2 (cluster 1), the elevation was 803 m while the average elevation in cluster 2 was 868 ± 63 m. In all plots included in the first two clusters the main soil type was *Calcaric Cambisol*, with inclusions of *Calcic Luvisol*. The soils of the plots included in cluster 2 had on average 18.9% clay, 34.8% silt and 46.4% sand, while in plot 2 the clay content was higher (29.8%). In cluster 3 (C3 in Fig. 3), the main soil types were *Calcaric Fluvisol* and the average elevation was 753 ± 12 m. This cluster grouped the plots in which soils had higher sand content (54.3% on average) and those with lower organic matter content (1.7%). In cluster 4 (C4 in Fig. 3), however, the main soil types were *Calcaric Cambisol* and *Calcaric Fluvisol* and the average elevation was 824 ± 33 m. Texture and organic matter of the soils included in cluster 4 were quite similar to those of cluster 2, with clay contents of 16.8% and sand contents of 27%, on average. The distance from the plots to the river was also different between clusters. The highest value corresponded to the plots included in cluster 2 and the minimum to those included in cluster 3.

Table 7 shows the average dates of the phenological stages for the plots included in each cluster. Phenological dates in the plots included in cluster 3 were advanced on average 2 or 3 days in relation to those included in cluster 2 for all stages. For some stages (I, L) there were also an advance in the plots included in cluster 3 in relation to those included in cluster 4. Significant differences between clusters in relation to the phenological dates are indicated in Table 7. This advance is partially accounted for by the differences in elevation. Significant correlations were found between phenological dates of the stages L and M and elevation (at 95% level), with correlation coefficients greater than 0.57. In addition, a significant relationship was also found between phenology and texture. The phenological response of the plant is inversely related to its vigor, in such a way that the soil organic matter and clay contents favor

Table 4
Mean values and standard deviation (m ± std) of climate characteristics in each year of the period analyzed (2003–2013): TmaxGS (maximum temperature during the growing period), TminGS (minimum temperature during the growing period), ndT0 (number of frost days), ndT30 (number of days > 30 °C), PGS (precipitation during the growing period); PHY (precipitation during the hydrological year), PBB (precipitation during the bud-break to bloom period), PBV (precipitation during the bloom to veraison period), PVH (precipitation during the veraison to harvest period), ETcGS (crop evapotranspiration during the growing period), WI (Winkler index), HI (Huglin index).

Year	TMaxGS (°C)	TMinGS (°C)	ndT0 (days)	ndT30 (days)	PGS (mm)	PHY (mm)	PBB (mm)	PBV (mm)	PVH (mm)	ETcGS (mm)	WI (gdd)	HI (gdd)
2003	24.6 ± 4.0	12.7 ± 6.5	59.5 ± 15.7	57.7 ± 9.0	184.7 ± 58.1	444.5 ± 75.7	79.5 ± 26.2	32.3 ± 14.3	72.9 ± 34.1	599.4 ± 16.5	1493 ± 158	2324 ± 137
2004	22.5 ± 3.7	11.6 ± 7.1	78.5 ± 19.4	42.8 ± 17.0	144.6 ± 46.2	347.8 ± 102.2	103.6 ± 50.5	32.3 ± 19.6	8.7 ± 7.8	551.5 ± 42.0	1271 ± 300	1980 ± 311
2005	24.3 ± 3.5	12.0 ± 7.7	99.8 ± 12.7	59.3 ± 10.6	81.7 ± 44.1	296.5 ± 181.6	42.6 ± 19.5	28.0 ± 21.5	11.1 ± 7.2	600.2 ± 28.0	1496 ± 219	2287 ± 199
2006	24.0 ± 4.1	12.3 ± 6.8	72.8 ± 11.6	52.8 ± 13.3	189.0 ± 73.0	472.4 ± 64.9	76.3 ± 47.2	82.2 ± 28.3	30.5 ± 7.4	584.0 ± 26.2	1565 ± 178	2279 ± 173
2007	22.4 ± 2.8	11.2 ± 6.5	82.8 ± 19.6	24.7 ± 5.4	275.8 ± 50.0	498.3 ± 74.0	165.8 ± 33.7	41.3 ± 9.0	68.7 ± 29.4	541.4 ± 23.5	1153 ± 154	1904 ± 139
2008	22.7 ± 2.5	10.9 ± 6.4	73.3 ± 20.1	36.3 ± 5.0	295.4 ± 57.5	362.1 ± 143.7	210.4 ± 38.3	44.0 ± 17.3	41.1 ± 15.9	549.2 ± 14.6	1149 ± 121	1925 ± 99
2009	24.5 ± 3.8	12.1 ± 6.9	79.5 ± 16.7	57.3 ± 6.8	122.3 ± 44.4	381.6 ± 155.7	68.2 ± 14.4	37.2 ± 30.2	16.9 ± 12.1	600.0 ± 18.3	1520 ± 126	2275 ± 99
2010	23.7 ± 3.2	11.9 ± 6.6	71.8 ± 14.2	48.8 ± 6.9	205.9 ± 69.0	450.8 ± 55.9	135.1 ± 54.2	33.0 ± 11.8	37.8 ± 13.5	573.1 ± 12.3	1329 ± 151	2126 ± 211
2011	25.1 ± 3.4	11.7 ± 7.3	55.2 ± 25.2	45.0 ± 10.2	134.7 ± 15.4	392.0 ± 66.1	93.7 ± 27.0	21.1 ± 12.3	20.0 ± 13.1	602.3 ± 17.0	1447 ± 129	2347 ± 102
2012	24.1 ± 3.2	11.2 ± 7.1	96.2 ± 19.5	47.2 ± 7.8	152.4 ± 19.8	276.5 ± 19.0	90.1 ± 18.0	30.8 ± 14.0	31.4 ± 8.7	676.0 ± 143.8	1321 ± 56	2134 ± 78
2013	22.4 ± 2.5	11.8 ± 7.1	99.0 ± 8.2	49.7 ± 5.5	205.3 ± 17.3	557.1 ± 40.0	125.9 ± 29.9	41.3 ± 25.4	52.6 ± 17.4	528.9 ± 120.3	1044 ± 443	1738 ± 565

Table 5
Average dates and standard deviations (days) of the phenology stages C, G, I, L and M observed at 20 plots in the Ribera del Duero area during 2004–2013.

Stage C Budburst	Stage G	Stage I Bloom	Stage L	Stage M Veraison
mean ± std	mean ± std	mean ± std	mean ± std	mean ± std
27-April ± 6.1	19-May ± 4.6	15-Jun ± 8.0	15-Jul ± 7.0	13-Aug ± 7.7

the delay in some phenological stages where I, L and M stages were advanced in the soils with higher sand content, while later dates were found in soils with higher organic matter and clay contents. These results may be driven by the water holding capacity of the soils with these characteristics.

Although on average some differences in phenology were observed between irrigated and non-irrigated plots, they were not statistically significant. Similarly the differences between the two main types of training systems (vertical trellis or goblet) were not significant. Nevertheless, the results indicated a slight delay in phenology in the non-irrigated vines in most of the stages. Higher variability was also seen between plots in the non-irrigated compared to the irrigated vines, in particular during the earliest phenological stages although at the end of the cycle the differences were lower. The differences among the non-irrigated plots were also greater in the driest years.

3.4. Grape quality variability

The 26 plots used for the grape quality analysis were distributed throughout the Ribera del Duero DO, in different soil types (Fluvisols, Cambisols, Regosols, Leptosols and Arenosols), according to the soil characteristics shown in Table 8. Grape quality parameters obtained during the period analyzed varied among plots, but also between years, due to the differences in climatic conditions. The average values obtained for each year are shown in Table 9, where these differences can be observed. Taking into account this variability, the analysis was performed by considering the three groups of years with different temperature and rainfall characteristics (wet, intermediate, dry), which gave rise to similar water availability.

The results of the PCA of the grape quality parameters analyzed, and soil properties (texture, organic matter and pH) for the three groups of years are shown in Fig. 4. The variables analyzed were classified into three groups related to acidic characteristics, anthocyanin and color characteristics, and berry weights. However, soil properties appeared in different components, indicating the lack of significant relationships between grape parameters and soil properties.

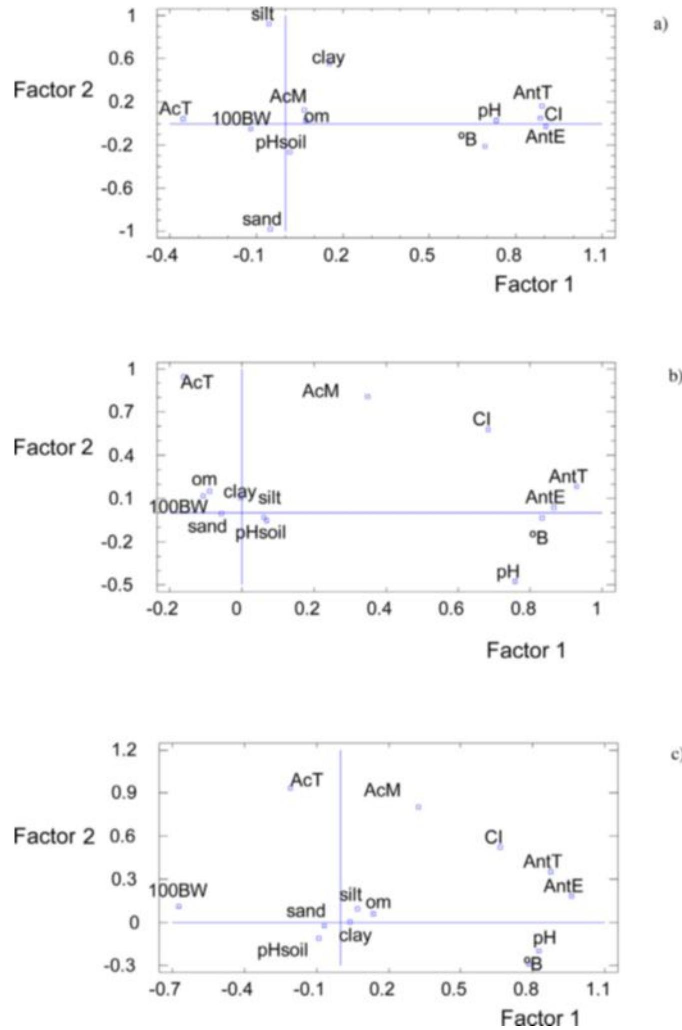

Fig. 4. Relationship between grape and soil parameters analyzed in the control plots in three groups of years with different characteristics. a) wet years; b) intermediate characteristics; c) dry years. (AcT: titratable acidity; AcM: malic acid; °B: soluble solids in °Baumé; 100BW; weight of 100 berries; AntT: total anthocyanins; AntE: extractable anthocyanins; CI: color intensity; o.m.: organic matter content).

The first component, which represented between 28 and 32% of the total variance, was related to color and anthocyanins, although pH and soluble solids were also correlated. The second component

Table 6
Soil type and average characteristics of the plots included in each cluster.

Cluster	Elevation (m)	Distance to River (m)	Plant Age (years)	Training System	Main Soil type	Clay (%)	Silt (%)	Sand (%)	o.m. (%)
1	803ab	1670ab	12	VT	Calcic Luvisol + Calcaric Cambisol	29.8a	34.9a	35.4a	1.5a
2	868 ± 63b	5648 ± 4397a	28 ± 15	VT ± G	Calcic Luvisol + Calcaric Cambisol	18.9b	34.8a	46.4a	2.1ab
3	753 ± 12a	891 ± 480b	21 ± 15	VT	Calcaric Fluvisol	22.2ab	23.5b	54.3b	1.7a
4	824 ± 33b	2810 ± 2400ab	27 ± 19	VT	Calcaric Cambisol + Calcaric Fluvisol	16.8b	36.2a	47.4a	2.5b

*Different letters mean significant differences at 95% level. (VT: Vertical trellis; G: Goblet; o.m.: organic matter)

Table 7
Mean dates and standard deviations (days) of the different stages for the plots included in each cluster during 2004–2013.

	Stage C	Stage G	Stage I	Stage L	Stage M
cluster 1	30-Apr	20-May	12-Jun	12-Jul	11-Aug
cluster 2	28-Apr ± 2.3a	20-May ± 2.2a	15-Jun ± 1.2a	15-Jul ± 0.8a	13-Aug ± 2.4a
cluster 3	26-Apr ± 2.1a	17-May ± 1.6b	13-Jun ± 1.4b	11-Jul ± 1.4b	10-Aug ± 1.0b
cluster 4	26-Apr ± 2.5a	16-May ± 1.5b	14-Jun ± 1.5ab	13-Jul ± 1.9c	10-Aug ± 0.5b

*Different letters mean significant differences at 95% level.

Table 8
Plot characteristics used in the ripening analysis.

Municipality	ID	Soil classification	Slope(%)	Elev.a.s.l.(m)	Distanceto river (m)	Age(y)	Training System
Quintanilla de Onésimo	1	Calcaric Fluvisol	4.5	739	604	25	VT
Olivares de Duero	2	Calcaric Fluvisol	1.0	725	451	25	VT
Valbuena	3	Calcaric Fluvisol	20.0	751	1179	65	B
Pesquera	4	Calcaric Fluvisol	11.0	765	1361	60	B
Pesquera	5	Lithic Leptosol + Calcaric Regosol	3.0	771	1854	60	G
Peñafiel	6	Lithic Leptosol + Calcaric Regosol	11.2	779	2218	18	VT
Peñafiel	7	Calcaric Fluvisol	9.5	780	2181	20	VT
Pedrosa	8	Calcaric Cambisol + Calcic Luvisol	3.5	855	5043	25	VT
Roa	9	Calcaric Cambisol + Calcic Luvisol	2.0	817	3221	60	G
Roa	10	Calcaric Cambisol + Calcic Luvisol	9.1	788	1104	22	VT
Olmedillo de Roa	11	Calcaric Cambisol + Calcic Luvisol	3.5	858	8999	55	G
La Horra	12	Calcaric Cambisol	9.0	831	3445	50	G
La Horra	13	Calcaric Cambisol + Calcic Luvisol	5.3	844	5503	25	VT
Gumiel	14	Calcaric Cambisol + Calcic Luvisol	9.0	826	8417	22	G
Gumiel	15	Cambic arenosol	4.7	808	3673	28	VT
Aranda	16	Calcaric Cambisol + Calcic Luvisol	4.0	869	1705	55	G
Aranda	17	Calcaric Cambisol + Calcic Luvisol	5.8	828	3822	20	VT
La Aguilera	18	Calcaric Cambisol + Calcic Luvisol	4.5	810	5658	48	G
Quintana del Pidio	19	Calcaric Cambisol + Calcic Luvisol	3.5	800	8927	50	G
Fuentelcesped	20	Calcaric Fluvisol	1.5	831	6103	60	G
Milagros	21	Calcaric Fluvisol	2.9	836	10868	58	G
Zazuar	22	Calcaric Cambisol + Calcic Luvisol	5.0	847	7867	60	G
Peñaranda	23	Calcaric Cambisol + Calcic Luvisol	4.3	916	4323	58	G
Castillo de Robledo	24	Calcaric Cambisol + Calcic Luvisol	3.7	892	3272	35	VT
San Esteban de Gormaz	25	Lithic Leptosol + Calcaric Regosol	8.3	880	1531	30	G
Atauta	26	Calcaric Cambisol + Calcic Luvisol	7.5	955	3984	68	G

VT: vertical trellis; G: Goblet

Table 9
Average values and standard deviations (26 plots) of each grape quality parameter evaluated in each year analyzed (AcT: titratable acidity expressed in g of tartaric acid; AcM: malic acid; °B: soluble solids, expressed in °Baumé; 100 BW: weight of 100 berries; AntT: total anthocyanins; AntE: extractable anthocyanins; CI: color intensity).

	pH	AcT (g/l)	AcM (g/l)	°B	100 BW(g)	AntT(mg/l)	AntE(mg/l)	CI
2003	3.60 ± 0.11	5.47 ± 0.45	3.48 ± 0.39	13.1 ± 0.8	179 ± 17	688 ± 187	283 ± 66	7.1 ± 1.1
2004	3.49 ± 0.10	6.30 ± 0.63	3.98 ± 0.58	13.1 ± 0.6	191 ± 25	933 ± 155	374 ± 55	6.9 ± 1.4
2005	3.69 ± 0.12	4.87 ± 0.39	3.27 ± 0.56	13.3 ± 0.7	148 ± 19	593 ± 110	242 ± 47	5.7 ± 1.6
2006	3.63 ± 0.11	5.01 ± 0.72	3.33 ± 0.62	13.0 ± 0.7	185 ± 15	570 ± 106	255 ± 56	5.5 ± 1.4
2007	3.51 ± 0.13	6.26 ± 0.96	4.38 ± 0.67	12.4 ± 0.7	185 ± 19	673 ± 163	270 ± 40	8.3 ± 1.8
2008	3.43 ± 0.11	6.89 ± 1.05	4.04 ± 0.50	12.1 ± 0.5	189 ± 24	662 ± 106	260 ± 40	8.5 ± 1.5
2009	3.62 ± 0.13	4.62 ± 0.69	3.48 ± 0.63	13.5 ± 0.7	177 ± 19	628 ± 98	259 ± 35	6.5 ± 1.8
2010	3.62 ± 0.10	5.93 ± 0.73	3.87 ± 0.57	13.6 ± 0.5	185 ± 24	690 ± 78	244 ± 34	8.8 ± 1.6
2011	3.64 ± 0.13	5.00 ± 0.59	2.63 ± 0.54	13.9 ± 0.7	180 ± 20	656 ± 86	291 ± 32	7.7 ± 1.2
2012	3.72 ± 0.14	4.54 ± 0.86	2.10 ± 0.87	13.1 ± 0.8	159 ± 26	564 ± 185	267 ± 69	7.9 ± 1.8
2013	3.60 ± 0.11	6.67 ± 0.79	3.58 ± 0.62	12.4 ± 0.6	184 ± 17	574 ± 78	275 ± 37	9.5 ± 1.7

represented between 18 and 22% of variance and was related to soil texture. The third component represented between 12 and 15% of variance and it was related to grape acidity (titratable acidity and malic acid). The fourth component represented 11% of variance and was related to soil organic matter and pH. Finally, the fifth component was related to the berry weights. The lack of correlation between grape and soil variables was confirmed in all groups of years analyzed.

Using these two main groups of variables (acidity and anthocyanins), the 26 plots were classified for each of the defined groups of years. The plots were classified into three groups according to anthocyanins and into two groups according to acidity. The average values for the plots assigned to each cluster for the three groups of years are shown in Tables 10a and 10b. The plots were classified in a similar manner for the three types of years (80%) and the rest were included in the same cluster in two of the three groups of years analyzed. Fig. 5 shows the plots included in each cluster when they were classified according to acidity and anthocyanin values. Regarding anthocyanin concentrations and color, significant differences were found between the wet and dry years. The synthesis of phenolic compounds can be affected significantly in situations of stress suffered by the plant in certain phases of the cycle (as in the case of the driest years), as well as adversely affected by the excess of growth and production favored by a higher water availability (in the case of the wetter years). Total anthocyanin values ranged between 507 and 661 mg/l in the dry years and between 540 and 723 mg/l in the wet years. However, the highest values were found in the years with intermediate characteristics (ranging between 666 and 758 mg/l). Similarly, the highest extractable anthocyanin concentrations were found in the intermediate year group, with values ranging between 253 and 311 mg/l, while in the wet years values ranged from 234 to 277 mg/l. In the dry years, however, the differences among the groups of plots established were greater (from 227 to 290 mg/l in the dry years). Similar behaviour was found for the color intensity values.

Furthermore, it was also observed that the lowest anthocyanin concentrations were recorded in the plots with the lowest grape pH. Significant differences between plots with similar behaviour in each group of years are indicated in Table 10a. The highest values of anthocyanin concentrations were observed in plots located at lower elevations and at shorter distances to the river (distances up to 2500 m and elevation up to 800 m a.s.l.).

The soluble solids concentrations were greater in the dry and in the intermediate years compared to the wet years. In the wet years the total soluble solids ranged between 11.9 and 12.3 °Baumé, while in the dry years and the intermediate years sugar levels ranged, between 12.8 and 13.6 °Baumé and between 12.9 and 13.5 °Baumé, respectively. Higher moisture conditions have associated greater

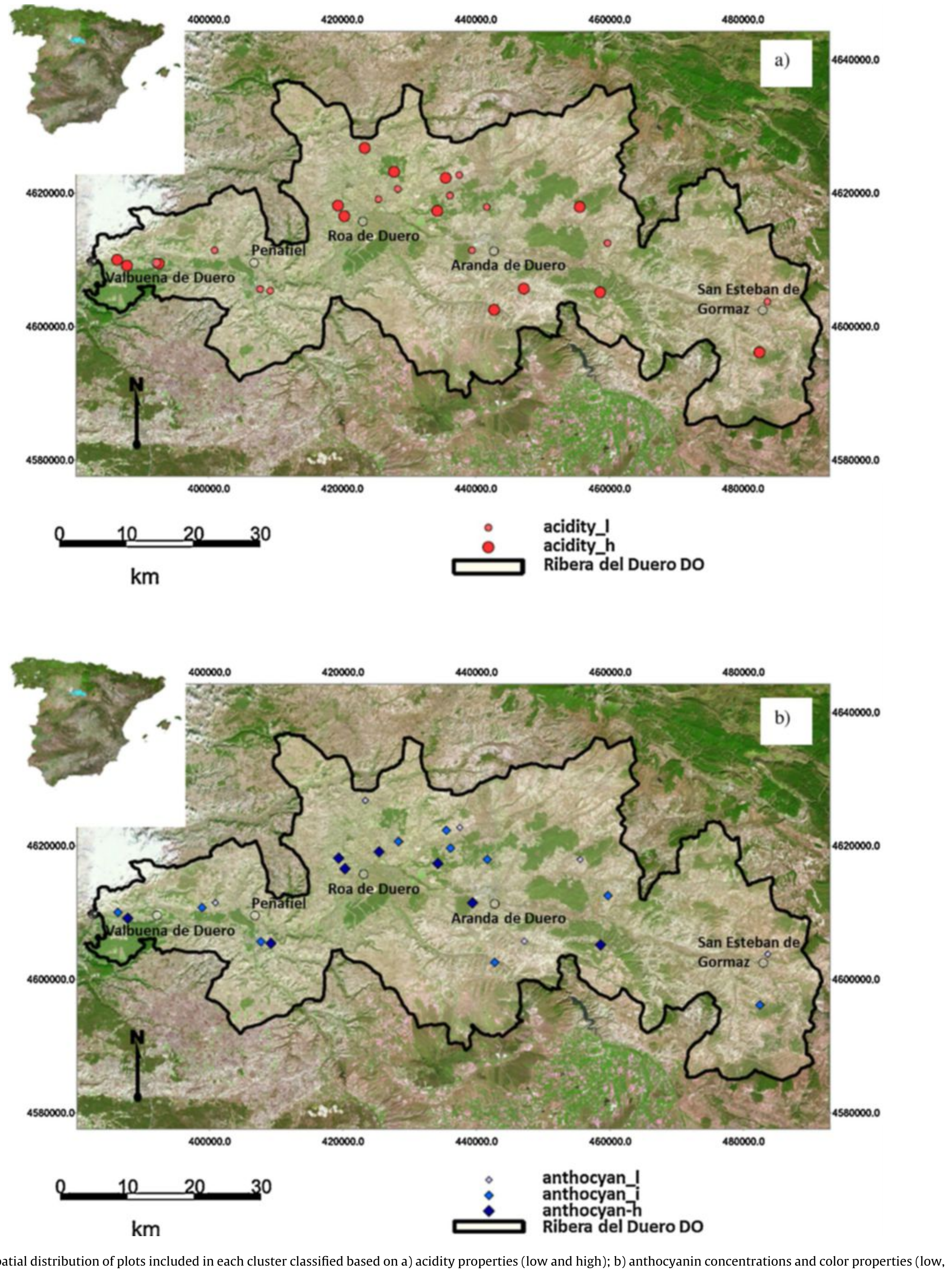

Fig. 5. Spatial distribution of plots included in each cluster classified based on a) acidity properties (low and high); b) anthocyanin concentrations and color properties (low, intermediate, and high).

Table 10a
Average values and standard deviations of each quality parameter for the plots grouped in the retained clusters based on anthocyanin characteristics (AntT: total anthocyanins; AntE: extractable anthocyanins; CI: color intensity; °B: soluble solids in °Baumé; 100 BW: weight of 100 berries; o.m.: organic matter content); (w: wet years; d: dry years; i: intermediate years).

Cluster	Grape pH	°B	AntT (mg/l)	AntE (mg/l)	CI	100 BW (g)	Clay (%)	Silt (%)	Sand (%)	o.m. (%)	Elev (m)	Dist. River (m)
w1	3.57 ± 0.14a	12.3 ± 0.5a	723 ± 90a	273 ± 34b	8.7 ± 1.3b	173 ± 15a	21.5 ± 6.6	35.7 ± 10.0	43.5 ± 15.2	2.04 ± 0.8	789 ± 40a	2530a
w2	3.36 ± 0.09a	11.9 ± 0.7a	540 ± 76b	234 ± 23a	6.9 ± 0.8b	204 ± 22b	21.5 ± 6.8	36.7 ± 6.6	40.6 ± 13.0	2.2 ± 1.2	831 ± 39b	5835b
w3	3.47 ± 0.11a	12.3 ± 0.5a	682 ± 89c	277 ± 34b	8.6 ± 1.4b	194 ± 20b	18.3 ± 7.6	37.2 ± 5.8	39.1 ± 10.9	1.7 ± 0.8	862 ± 45b	5308b
d1	3.68 ± 0.14a	13.6 ± 0.5a	661 ± 43c	290 ± 13b	8.9 ± 0.9b	166 ± 9a	25.2 ± 10.9	333.8 ± 8.6	41.1 ± 15.2	2.1 ± 0.7	803 ± 40a	2472a
d2	3.50 ± 0.09 b	12.8 ± 0.7b	507 ± 20b	221 ± 30a	6.3 ± 1.2a	184 ± 12b	31.8 ± 11.6	31.7 ± 13.0	36.5 ± 20.5	2.5 ± 0.8	818 ± 39a	4149a
d3	3.71 ± 0.11 ac	13.2 ± 0.5c	616 ± 33c	275 ± 12b	7.2 ± 0.5b	164 ± 10a	21.0 ± 11.0	36.5 ± 10.0	42.4 ± 16.1	1.8 ± 0.9	855 ± 45b	6291b
i1	3.71 ± 0.07 a	13.3 ± 0.4a	758 ± 32c	311 ± 34b	6.7 ± 1.0ab	176 ± 10a	21.3 ± 9.1	33.5 ± 10.8	45.2 ± 15.0	2.03 ± 0.6	787 ± 40	2542a
i2	3.52 ± 0.09 b	12.9 ± 0.4a	666 ± 56b	253 ± 31a	5.9 ± 0.8a	187 ± 11b	21.5.9 ± 14.0	35.9 ± 5.6	37.32 ± 1.2	2.2 ± 0.8	833 ± 39a	5826b
i3	3.57 ± 0.06 b	13.1 ± 0.5a	730 ± 55c	297 ± 23b	6.8 ± 0.9ab	199 ± 10b	18.5 ± 9.1	35.1 ± 8.9	46.8 ± 15.8	1.7 ± 0.9	875 ± 45b	5114b

*Different letters mean significant differences at 95% level.

Table 10b
Average values and standard deviations of each quality parameter for the plots grouped in the retained clusters based on acid characteristics (AcT: titratable acidity in g of tartaric acid; AcM: malic acid; 100 BW: weight of 100 berries; o.m.: organic matter content); (w: wet years; d: dry years; i: intermediate years).

Cluster	Grape pH	AcT (g/l)	AcM (g/l)	100 BW (g)	Clay (%)	Silt (%)	Sand (%)	o.m. (%)	Elev (m)	Dist. River (m)
w1′	3.6 ± 0.1	5.6 ± 0.7a	3.9 ± 0.4a	183 ± 18a	21.3 ± 8.1	37.5 ± 6.0	41.2 ± 8.9	2.2 ± 0.8	822 ± 60	3295a
w2′	3.4 ± 0.1	7.0 ± 0.6b	4.5 ± 0.5b	185 ± 21b	27.7 ± 7.4	31.7 ± 11.0	40.6 ± 16.0	2.2 ± 1.0	823 ± 52	4647b
d1′	3.7 ± 0.1	4.9 ± 0.3a	2.7 ± 0.2a	171 ± 13a	22.3 ± 7.6	34.7 ± 11.7	42.9 ± 13.5	2.1 ± 0.6	820 ± 60	3600a
d2′	3.7 ± 0.1	5.5 ± 0.4b	3.3 ± 0.3a	168 ± 13a	28.1 ± 12.0	33.2 ± 7.7	38.7 ± 15.0	2.1 ± 0.9	825 ± 52	4654b
i1′	3.7 ± 0.1	5.1 ± 0.2a	3.3 ± 0.4a	183 ± 9a	21.3 ± 6.5	38.4 ± 6.4	40.3 ± 9.0	2.0 ± 0.7	822 ± 60	3299a
i2′	3.6 ± 0.1	5.7 ± 0.4b	3.8 ± 0.3a	186 ± 12b	28.2 ± 11.5	30.9 ± 9.8	40.9 ± 16.0	2.2 ± 0.9	823 ± 52	4647b

*Different letters mean significant differences at 95% level.

water availability for the vines, which tend to show greater vegetative development and production. This fact typically involves a higher production of sugars, but its concentration in the grape can be lower.

Regarding the classification based on acidity characteristics, the differences between the two established groups for titratable acidity and malic acid were greater in the wet than in the dry or intermediate years. The titratable acidity ranged between 5.6 and 7.0 g/l in the wet years while in the other groups it ranged between 4.9 and 5.5 g/l, and between 5.0 and 5.7 g/l, respectively. The malic acid levels ranged between 3.9 and 4.5 g/l in wet years and between 2.7 and 2.8 g/l in the rest of years. No significant differences among groups were found in the average pH. It is known that greater water availability to the vine generates higher acid concentration in the grape (Sebastian et al, 2015; Luciano et al., 2013) while water deficits reduce acidity as berries contain less malic acid (van Leeuwen et al., 2004). In the case of this study, the highest acidity values were found in the plots located near the river (distances up to 2500 m and elevation up to 800 m a.s.l.), whose soils may have greater soil moisture conditions.

The 100 berry weights varied between 164 and 204 g, being higher in the wet years and intermediate years compared to the dry years. This result was consistent with the fact that berry size is dependent on vine water status. Berry weights, which did not exhibit correlations with other parameters in the PCA analysis, was slightly higher in the plots with lower grape pH and in those where the lowest anthocyanin concentrations and lower color intensity was observed. Among soil characteristics, clay, sand and organic matter contents showed differences among the groups of plots, although they were not statistically significant. Despite the lack of relationships between grape composition variables and soil characteristics observed in the PCA, some relationships were found when the plots were separated in the clusters (Table 10b). The highest acidity values in grapes were recorded in soils with slightly higher clay contents. The amount of acid and the acid concentration of the grape are usually favored by the vigour of the vine and it is foliar development, which tend to be higher in soils with a higher clay and organic matter content. These plots were located on the hillsides above the river terraces. Additionally, the highest anthocyanin concentrations and color intensities were found in plots with slightly higher sand content. These plots also had the lower berry weights. Nevertheless, the differences in soil characteristics among groups were not significant. Regarding the soluble solids, there were only differences between clusters in the dry years, with higher values in the soils with higher sand content.

4. Discussion

4.1. Phenology and soil properties

In the present study, the spatial variability in phenology and grape quality within the Ribera del Duero DO was evaluated. The effect of soil type and plot characteristics were considered in the interpretation of the observed differences during 2003–2013 in the region. In addition to the differences in the phenological dates due to the different climatic conditions recorded among years, differences in phenological dates were observed between the eastern and the western parts of the DO area and between elevations of the plots. The cluster analysis produced the separation of the plots in these portions of the DO and at different elevations. The results confirmed that earlier phenological dates from stage G to the end of the growth cycle were seen at lower elevations. This result agrees with Falcao et al. (2010) examining Cabernet Sauvignon in Brazil, who related the later phenology and longer duration of phenological events with an increase in elevation, and linked the lower and higher elevation to the warmer and cooler climate conditions, respectively.

In relation to soil characteristics, soil depth, texture and structure and their influence on soil moisture content appeared to have the greatest influence on vine development. The plots with soils that had higher percentage of clay, exhibited a later response in phenology in almost all phenological stages. Clay soils tend to hold more moisture and are often cooler, resulting in a delay in phenological timing. On the other hand, the plots located on the river terraces exhibited earlier phenological dates. In these areas the main soil type is Fluvisol with stratified profiles formed from fluvic material and with medium to coarse texture. The lower water holding capacity of these soils, with a higher percentage of sand, could explain the earlier phenological response. On lighter soils, with higher sand content, vines have a lower vegetative development and it is generally associated with an earlier phenology. Finally, the group of plots located in the hillsides above the river terraces with soils classified as *Calcaric Cambisols* and *Fluvisols* gave an intermediate response. In this group, soil characteristics were similar to those of the group 2 but with slightly higher organic matter content. However, other plot characteristics such as elevation and distance to river were different.

The results are in agreement with those of Trought et al. (2008) who related the influence of soil texture on phenology, indicating that the higher the proportion of gravelly soils, the more advanced the vine phenology. These authors attributed this effect in soils with gravels that outcrop to the surface, to a higher soil temperature when compared to deeper silty soils. Other research has also confirmed the effects of soil characteristics on grape development in different regions of France and Spain (van Leeuwen and Seguin, 1994; Morlat and Jacquet, 1993; Tardáguila et al., 2011).

In addition, according to Smart and Coombe (1983), the available moisture per soil unit depth varies from 30 mm m^{-1} in sands to 160 mm m^{-1} for clays, and the effect of clay content on the soil water available for vine roots and the corresponding plant water status have been also highlighted by Bodin and Morlat (2006). In this case, a measure of available water for the crop estimated by precipitation minus evapotranspiration during the growing season provided a first approximation of the influence of water availability on phenological timing.

4.2. Grape quality and soil properties

In this study soil physical characteristics did not exhibit significant correlations with grape quality parameters when the analysis was performed with all plots together. However, when they were separated according to their acid and anthocyanin values some relationships were observed. In the Ribera del Duero, the plots whose soils had higher percentage of clay and delayed phenology also showed the lowest grape pH values and higher titratable acidity in the three situations (wet, intermediate and dry years). In addition the higher anthocyanin concentrations and the lower berry weights were found in soils with the highest sand content. This result, which could be associated with the water holding capacity of these soils, is in agreement with the results of Böhm (2013). On the contrary, the plots located in the river terraces (*Fluvisols*), which exhibited earlier phenological dates, had the highest soluble solids values, lower anthocyanins and color intensity, along with the lowest berry weights. The plots located in the areas on the hillsides above the river terraces with high sand and clay contents (in a mix of *Calcaric Cambisols* and *Fluvisols*) gave intermediate values for all parameters. The observations that clayey soils tend to exhibit greater vegetative development would ultimately act to reduce berry exposure to sunlight and lower the air flowing through the canopy. The result is that the vines growing on clayey soils would tend to have slower physiologic activity, later phenology, slower ripening, greater disease pressure and have minor reductions in the tartaric acid and, above all, in malic acid. Ultimately the maturing fruit would have a more reduced pH and a higher total acidity for a certain level of sugars at the time of harvest.

Coarse texture influences soil temperature and evapotranspiration and tends to produce higher alcohol contents in wine (Böhm, 2013). The influence of sandy soils in the quality of the grapes and the wine reflects a balance of the vineyard in favor of the fruit, to the detriment of vegetative growth (van Leewen et al., 2004; De Andrés-de Prado et al., 2007; Gómez-Miguez et al., 2007; Renouf et al., 2010; Priori et a., 2013 De Andrés-de Prado et al., 2007; Gómez-Miguez et al., 2007 Renouf et al., 2010). In general, soils with better drainage (sandy) tend to produce the best wines, with more balanced acidity, lower and smoother tannins in comparison with high clay content soils which are usually too deep and potentially very fertile and often considered unsuitable for viticulture. Silty soils often have negative effects on chemical and physical properties (Böhm, 2013), although some authors found loamy soils especially suitable for producing quality wine grapes in some valleys of British Columbia (Bowen et al., 2005).

In addition soil water also affects acidity, alcohol and tannin content where wet soils give rise to high acidity, high tannin content and low alcohol (Böhm, 2013). The results observed in the study area agree with those found in previous research. Amenta and Buondonno (2012) indicated that flavor, tartaric acid, malic acid and titratable acidity of grapes were significantly dependent on soil features such as the fineness of the texture, neutral to alkaline pH and an appreciable content of soil organic matter and high cation exchange capacity. Trought et al. (2008) found some influences of soil texture and soil depth on pH and tartaric acidity in New Zealand vineyards. Their results indicated that the higher the proportion of gravelly soils the riper the fruit, while there was lower acidity and higher pH in shallow soils compared to deeper soils. However, they did not find significant effects on fruit yield.

The lower berry weight in soils with low water holding capacity has been indicated in other studies (Ubalde et al., 2010), which is consistent with a reduction in berry weight and yield caused by low water supply to the vines (Peyrot des Gachons et al., 2005; van Leeuwen et al., 2003). The observed higher values in anthocyanin concentrations found in the soils with high sand content and lower water holding capacity in this study agree with the results found by De Andrés-de Prado et al. (2007) for Grenache. Their research indicated that wines produced on soil with higher water holding capacity resulted in significantly lower color intensity and phenolic composition. Similarly, Cheng et al. (2014) found higher anthocyanin concentrations in Cabernet Sauvignon grape skins from the soils with less water and organic matter. The results seen in Tempranillo in the Ribera del Duero also corroborated the hypothesis from García Navarro et al. (2011), that indicated that the delay in ripeness observed in vineyards planted in Luvisols with high depth and water-holding capacity, may improve the biosynthesis and accumulation of phenolic and aromatic compounds. This influence has also been pointed out by Jackson and Lombard (1993) and is likely enhanced by cooler nights during ripening.

The effect of soil water holding capacity was also evident in the soluble solids and the potential alcohol levels, with highest values recorded in soils with higher sand contents and lower water holding capacity, similarly to that found by Ubalde et al. (2010). The highest values also corresponded to the driest conditions in which greater water stress were recorded, in agreement with results found by Reynolds and Naylor (1994).

Leone et al. (2010) found that soil and climate independently affect quantitative and qualitative grape features, respectively. They indicated that the structure of the relationships between soil and grape variables was highly comparable and consistent from one year to another, while the values of grape compounds, such as tartaric and malic acids and titratable acidity, soluble solids and must pH varied significantly from year to year of the period they analyzed. However, the mean weight of clusters and berries did not change during the time period. The variation in the individual berry weight tends to be less sensitive to the interannual variation in climatic conditions compared to the cluster weight as a whole in the same vineyard when crop operations are consistent each year. It has been observed in many cases that the number of flowers and, above all, the number of berries per cluster is the component of yield most sensitive to the interannual variations.

On the other hand, Luciano et al. (2013) found that the physical-chemical characteristics of some varieties (Cabernet Sauvignon in their study) were more affected by weather than by soil type. They found that years with lower rainfall and higher temperature ranges favored greater accumulation of soluble solids in the Cabernet Sauvignon grape and that years with higher rainfall favored higher retention of acidity. This result was also confirmed in the Ribera del Duero with the grape characteristic observations from years with differences in rainfall (Table 10a and b). The highest soluble solids values were recorded in the driest years, while those years recorded the lowest acidity levels (both tartaric and malice acids). The highest total and extractable anthocyanins were recorded in wet and intermediate years, while in the driest years the levels were the lowest. This result differs from that of Ubalde et al. (2010) who found higher values in the driest years, although they indicated that the lower values were found in the hottest years they analyzed. In our case, the climate characteristics of the region showed that the driest years were also the hottest during 2003–2013, in which up to 70 days with more than 30 °C were recorded during the growing season. In this respect, previous studies have indicated the negative effect of high temperatures on anthocyanin accumulation (Kliewer, 1970). On the other hand, Falcao et al. (2010) highlighted the effect of cooler conditions, especially from locations at higher elevations, on favoring color development. In the Ribera del Duero case, however, the highest anthocyanin and color indexes were found in the plots located at lower elevations on the river terraces, while the lower values were recorded in vineyards located on the hillslopes at higher elevations. The differences in behaviour could be due to the microclimate created by the river more than to the effect of elevation. In addition, Piretti et al. (1976) and Fregoni (1977) indicated that grape phenolic composition is greater in years with higher precipitation; and Freeman et al. (1979) and Guilloux (1981) found less color in vineyards with higher water availability, which may be mainly due to the greater berry size reached in those conditions. In the Ribera del Duero case, however, the highest anthocyanin concentrations were found in the intermediate years, which had in common very low precipitation between veraison and harvest (18 mm on average), while in other years the amounts were nearly twice as much. This could be the reason of the higher anthocyanins concentration in those years. Furthermore, the concentration of the various phenolic compounds during ripening in the Tempranillo variety is very sensitive to heat stress situations or, conversely, to excess water availability. These sensitivities could justify the fact that in this research the years higher in anthocyanin concentrations and phenolic compounds were those with moderate climatic conditions, in which no excessive stress nor excess of rainfall occurred.

5. Conclusions

This analysis has helped establish the spatial and temporal characteristics and variability in phenology and grape ripening within the Ribera del Duero region. Furthermore, the research has documented some of the observed influences between landscape and soil characteristics and phenological timing and fruit quality parameters. Average differences of 2 or 3 days can be found for most of the phenological stages throughout the growth cycle between the western and eastern parts of the area. Differences in phenology were also found between areas located at different elevations within the region. Grape quality parameters did not show a clear spatial pattern, but some properties, in particular those that control plant water availability, affected the values of these parameters, with higher acidity in soils with greater clay and organic matter contents. Regarding anthocyanin concentrations, the results show that levels in the ripening fruit are highly dependent on whether conditions are wet or dry. Higher anthocyanin concentrations were observed in the soils with greater sand content which were located at lower elevations. Thus, despite the variability of grape quality parameters associated with climatic conditions, soil type contributed to spatial variations in grape quality, in particular acidity, anthocynanins and color. The variability in phenology and ripening characteristics found within the Ribera del Duero DO, related to soil and plot characteristics, highlights the possibilities of establishing viticultural zones that produce different vine responses and different qualities of fruit harvested. Which in turn may allow for the establishment of more exacting differences in vineyard treatments and management and the elaboration of site specific wine styles.

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