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Variability in grape composition and phenology of 'Tempranillo' in zones located at different elevations and with differences in the climatic conditions

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Summary

The objective of this research was to analyse the variability on phenology and berry composition of 'Tempranillo' among different elevations and different climatic conditions. The study was conducted on 'Tempranillo' vineyards located in Rioja DOCa at elevations between 367 and 650 m a.s.l. Phenology and the evolution of berry composition during ripening, related to pH, total acidity, malic acid, total anthocyanins, total polyphenols index, colour intensity and sugar content were analysed for the period 2008-2018 at each elevation. The weather conditions recorded during the period of study were evaluated.

The results indicate that, on average, differences between the analysed elevations of up to 21 days existed in the dates in which the maturity was reached, being the differences higher in the years with extreme conditions (very wet or dry and very hot or cool). The comparative analysis among elevations and in years with differences in temperature and in precipitation allowed knowing the differences in the vine response under different climatic conditions. From this analysis some conclusions may be extracted that may be useful for police makers to define new planting areas in which less imbalanced situations may occur under warm conditions.

K e y w o r d s : acidity; anthocyanins; climatic characteristics; ripening; spatial and temporal variability.

Introduction

Climate together with soil characteristics and topography are part of the elements of the "terroir effect" (Reynard *et al.* 2011, Van Leeuwen *et al.* 2004) and play an important role on grapevine growth and berry composition. Each variety has its own phenological timing due to its own morphological and physiological characteristics, and growing season climatic characteristics define the suitability of each variety to be grown in a given zone. Each variety can be cultivated in a given temperature range (Jones 2012) as temperature affect grapevine development and growth as well as several grape composition (Webb *et al.* 2012, Sadras and Moran 2013, Ovadia *et al.* 2013).

Temperature affect sugars, acidity, phenolic compound development and its concentrations in the berries, as well as aroma and flavours (Giomo *et al.* 1996, Greer and Weedon 2013, Sadras and Moran 2012, Bonada *et al.* 2015). The temperature levels at which the sugar enzymes are active (8 to 33 °C) are different to the ones for the activity of the colour enzymes (17 to 26 °C) (Iland and Gago 2002). It is generally thought that high temperatures could negatively affect the anthocyanin accumulation (Mori *et al.* 2005, 2007, He *et al.* 2010, Mira de Orduña 2010) probably because, under warm conditions, the activity of some key enzymes is likely to be inhibited (Mori *et al.* 2005) and anthocyanin degradation tends to occur (Mori *et al.* 2007). In areas with high temperatures, grapes tend to have lower total acidity and a higher pH (Keller 2010). The accumulation of malic acid mostly occurs before veraison and the optimum temperature range for the accumulation is between 20-25 °C; when temperatures are more than 38 °C, the synthesis declines greatly (Keller 2015). Like the case of tartrate, malate accumulation also tends to decline under water deficit (Esteban *et al.* 1999). However, other climatic variables such as water inputs and solar radiation play an important role on grape composition and on colour and aroma (Gregan 2012, Sebastian *et al.* 2015).

On the other hand, topography influences also viticulture in a given region. In this respect, both elevation and slope are important factors to consider. The variation of temperature with elevation due to the vertical temperature gradient may drive the optimal cultivar in a given place and the slope degree of the terrain impacts on canopy microclimate, water intake and drainage (Fraga *et al.* 2014) and also water losses by runoff. In addition, topography also affects vine management (Costantini *et al.* 2016).

Differences in the vine response at different elevations already indicated by other authors in other viticultural areas. Failla *et al.* (2004), in mountain viticulture found elevation as one of the factors that influenced budbreak and bloom dates. Falcao *et al.* (2010), examining 'Cabernet Sauvignon' in Brazil, related the later phenology and longer duration of phenological events with an increase in elevation. Ramos *et al.* (2015a) found differences in the phenological dates for 'Tempranillo' between plots located at different elevations in the DO Ribera del Duero (Spain), indicating earlier phenological dates from stage G to the end of the growing cycle

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at lower elevations. Costantini *et al.* (2016) found specific landscape characteristics (including morphology, elevation and slope degree of the terrain) as factors that significantly influence the wine economy structure of a country.

In addition, soil physical and chemical characteristics are important factors that control vine development and fruit or wine quality. Soil water content influences vine water status, which impact berry growth and phenolic composition (Deloire *et al.* 2005), and soil mineralogy influence wine quality (van Leeuwen *et al.* 2004).

The 'Tempranillo' variety, which was originated by a spontaneous hybridization between the varieties 'Benedicto' and 'Albillo Mayor' (Ibáñez *et al.* 2012), is the third most cultivated wine variety in the world, after 'Cabernet Sauvignon' and 'Merlot', with a vineyard area of 230,000 hectares. Its name comes from the Spanish "temprano" meaning "early" and it does in fact ripen quite early (Martínez de Toda, 2018). It is the most cultivated variety in Spain, which is the country that contributes with the highest cultivated surface of this variety in the world (about 89 %), being Rioja the main producer area. This variety is very versatile from an oenological viewpoint. With respect to agronomic performance, it sets well but is not very resistant to drought or to high temperatures

The acidity levels of 'Tempranillo' are usually low, with a tendency towards higher pH. These characteristics are due in part to their ability to accumulate potassium in the berries with the result of a decrease in the total acidity of the wine and an increase in pH. Therefore, its cultivation is more suitable in cool areas, where the wines preserve a higher level of acidity and have a greater balance in its composition (Balda and Martínez de Toda 2017). In Spain, 'Tempranillo' is cultivated at elevations that range between the sea level and 1,300 m a.s.l, and in a high range of temperatures, even overpassing the optimal limits suggested by Jones (2012). In other viticultural areas, in which this variety has been more recently introduced, the range of altitude and climatic conditions at which this variety is cultivated is also high; for example, it is grown at 3,000 m a.s.l. in the highest vineyard in the world, in Salta, Argentina.

The Rioja DOCa is the largest denomination of origin, of red wine, in Spain and, in addition, it is one of the only two denominations with the highest qualification (DOCa). The total vineyard area is about 65,000 ha, of which about 52,000 correspond to the 'Tempranillo' variety. The vines are cultivated from the terraces of the Ebro river to elevations up to about 700 m a.s.l. The climatic conditions, with Atlantic and Mediterranean influence, allow to separate different areas, which are recognized as Rioja Alta (about 27,000 ha) with Atlantic influence, Rioja Alavesa (about 13,000 ha) with intermediate climatic influence and Rioja Oriental (about 24,000 ha), with Mediterranean influence. Thus, within this viticultural area, there is a high range of conditions, from whose analysis conclusions can be drawn to other viticultural areas of the world where this variety can be cultivated.

The objective of this research was to investigate the differences in phenology and grape composition of 'Tempranillo' variety among zones located at different elevations, which affect the climatic conditions, and taking into account also soil properties that can affect vine development. The differences in the response under cooler and warmer areas in years with different climatic conditions may be used to establish strategies for selecting the best areas to maintain grape quality in the 'Tempranillo' variety.

Material and Methods

Data and analysis: Six vineyard plots located in Rioja Alta and Rioja Oriental, which were planted with the 'Tempranillo' variety, were selected in order to evaluate phenology and grape composition along the period 2008-2018. The plots selected in Rioja Alta were representative of two zones with different climatic characteristics: two plots were located in the municipalities of Haro and Cenicero (zone denoted as RA1) and another two plots were located in Sotés and Alesanco (denoted as zone RA2). In Rioja Oriental (RO), two additional plots were selected, which were in the municipalities of Alfaro and Aldeanueva de Ebro (zone denoted as RO). The plots were located at 465, 450 m a.s.l. in RA1, at 635 and 650 m a.s.l in RA2 and at 376 and 396 m a.s.l. in RO (Suppl. Fig. S1). Vines were planted between 1993 and 1999; they were bush trained and mainly cultivated under rainfed conditions, with 3,000 to 3,500 vines·ha^{-1} and with a yield below 6,500 kg·ha^{-1}.

Soil properties: Soil characteristics of the study plots related to soil organic carbon, soil particle distribution (clay, sand, silt and coarse elements) and soil water retention corresponding to field capacity and wilting point were obtained from the European Soil data base (ESDAC). The results are shown in Tab. 1. The soils of the studied plots have clay contents that range between 21.1 and 29.7 %; silt contents that range between 38.4 and 49.6 % and sand contents that ranged between 27.9 and 39.4 %. The organic carbon content varied between 0.60 and 1.53 %. The water retention corresponding to field capacity ranged between 25.2 and 27.9 % and that for the wilting point ranged between 15.3 and 17.1 % (Tab. 1). All soils have loamy texture, and the differences among them are small. Thus, climate and elevation could be considered as the main factors that drive the differences in the vine response.

Vine phenology and grape composition: For each zone, phenology was evaluated for the period 2008-2018. Two phenological stages (H and M), defined according to Baillod and Baggiolini (1993), whose dates were available for all plots and years studied, were analysed. The phenological date for each stage was defined taking into account the date at which at least 50 % of plants in the plots had reached the specific stage. Maturity was defined based on the date in which a given probable volumetric alcoholic degree (PVAD) was reached. The information was provided by the Consejo Regulador Rioja DOCa. Additional information related to budbreak, and flowering was obtained from previous works carried out in the area of study (Martínez de Toda and Balda 2015, Pérez, 2016, Zheng *et al.* 2017a, b and c). This information was used to estimate the average climatic conditions of different periods in each area.

Grape composition during ripening, analysed weekly, were evaluated for the same period (2008-2018). Parameters

Table 1

Soil properties of the analysed plots (RA1 and RA2: zones located in Rioja Alta; RO: zone located in Rioja Oriental)

Plot	Elev (m)	Clay (%)	Silt (%)	Sand (%)	Coarse elements (%)	OM (%)	FC (%)	WP (%)
RA1-P1	465	22.2	38.4	39.4	14.2	1.00	26.1	15.3
RA1-P2	450	25.5	45.3	29.2	13.0	0.75	27.0	16.2
RA2-P1	635	24.4	44.3	31.3	10.7	1.10	27.0	17.1
RA2-P2	650	29.7	42.4	27.9	17.1	0.60	27.9	17.1
RO1-P1	367	21.1	49.6	29.3	10.3	1.53	26.1	15.4
RO2-P2	379	21.4	44.9	34.7	15.5	1.45	25.2	15.3

such as pH, total acidity, malic acid (AcM), total anthocyanins (AntT), total polyphenols index (TPI), colour intensity (CI) and sugar content (expressed as probable volumetric alcoholic degree (PVAD) were evaluated. All analysis were done following the methods recommended by the OIV. The evolution of grape composition during ripening recorded in each plot along the period was evaluated and related to climate characteristics.

Climate data: The climatic conditions were analysed for the same period using the information recorded in meteorological stations located near the analysed plots, which belongs to La Rioja Government (Suppl. Fig. S1). Data from the meteorological stations of Haro, Uruñuela, Villar de Torre, Alfaro and Aldeanueva de Ebro were analysed. Daily maximum and minimum temperatures, precipitation, solar radiation, relative humidity and wind speed were analysed. Additional indices such as crop evapotranspiration and the Winkler index (accumulated degree days (base 10 °C, April-Oct), the number of days with extreme temperatures (T < 0 °C and T > 35 ºC) and precipitation recorded during the growing season, were calculated for each station. In order to analyse the effect of climate on grape composition, each analysed parameter was related to temperature and precipitation variables relative to the growing season and to periods between the different phenological dates, using a stepwise multiple regression analysis. The analysis was done separately for each zone. The Statgraphics package was used for the statistical analysis.

Results

Weather conditions recorded during the period of study: Years with different climatic characteristics were recorded during the period under study, with some differences between the three analysed zones (Tab. 2). The mean growing season temperature (TmGS) ranged between 15.9 and 18.0 °C in RA1, between 14.7 and 16.5 °C in RA2 and between 17.0 and 19.4 °C in RO. The maximum growing season temperature (TmaxGS) varied between 20.8 and 26.1 °C in RA and between 24.2 and 27.2 °C in RO, while the minimum growing season temperature (TminGS) ranged between 9.4 and 12.0 °C in RA and between 11.1 and 13.0 °C in RO. The average WI during the period under study was 1515, 1199, and 1800 GDD, respectively, in RA1, RA2 and RO, which means that the

Table 2

Average growing maximum, minimum and mean temperatures and number of days with extreme temperatures in each of the three zones analysed (TMaxGS: average maximum temperature during the growing season; TminGS: average minimum temperature during the growing season; TmGS: average mean temperature during the growing season; ndT0: number of days with T < 0 °C; ndT35: number of days with T > 35 °C; WI: Winkler Index; PGS: precipitation during the growing season). (RA1 and RA2: zones located in Rioja Alta; RO: zone located in Rioja Oriental)

	RA1							RA2							RO						
	Tmax GS	Tmin GS	Tm GS	WI	ndT0	ndT35	PGS	Tmax GS	Tmin GS	Tm GS	WI	ndT0	ndT35	PGS	Tmax GS	Tmin GS	Tm GS	WI	ndT0	ndT35	PGS
2008	22.9	10.4	16.0	1307	32	1	408.8	21.4	9.8	15.0	950	32	0	456.6	24.3	11.1	17.0	1509	46	4	353.2
2009	25.2	11.8	17.8	1606	38	10	129.7	22.8	10.2	15.9	1279	34	1	196.1	26.5	12.7	19.3	1907	36	17	167.2
2010	24.0	11.1	17.1	1437	35	6	155.4	21.7	9.6	15.3	1137	50	1	244.7	25.5	12.2	18.6	1725	50	12	122.1
2011	25.6	11.6	17.9	1594	26	9	109.0	23.0	10.4	16.0	1225	17	1	257.0	26.6	12.2	19.0	1871	30	6	148.1
2012	24.5	11.1	17.1	1550	31	15	233.2	22.3	9.7	15.4	1244	33	6	374.9	26.1	12.2	18.8	1840	41	20	244.0
2013	22.8	10.7	16.2	1353	34	4	238.4	20.8	9.5	14.7	1101	30	1	310.7	24.6	11.5	17.8	1643	40	5	269.4
2014	24.6	11.6	17.3	1539	22	3	186.1	22.3	10.3	15.7	1220	15	1	249.7	25.8	12.7	18.9	1830	24	3	241.4
2015	24.9	11.6	17.5	1527	31	12	144.9	22.8	10.3	16.0	1241	27	2	189.0	26.5	12.5	19.2	1886	29	20	166.5
2016	24.5	11.3	17.1	1546	25	16	98.7	21.8	9.4	15.1	1169	24	1	156.6	25.7	12.0	18.5	1811	27	17	177.0
2017	26.1	11.7	18.0	1661	23	16	144.8	23.8	10.5	16.5	1367	24	2	255.0	27.2	12.5	19.4	1907	29	17	202.5
2018	24.7	12.1	17.6	1544	29	5	212.7	22.4	10.7	15.9	1256	23	1	378.2	26.5	13.0	19.3	1867	23	10	289.9

studied zones are classified as Region II (RA1), Region I (RA2) and Region III (RO).

Another factor that may affect vine development and grape composition is the occurrence of extreme temperatures. In the present research, it was observed that the number of warm extremes (number of days with T > 35 °C - ndT35) was significantly smaller in RA2 than in RA1 and RO while the number of cool extremes (number of days with T < 0 °C -ndT0) was significantly different in the three zones with the smallest values in RO and the highest values in RA1. During the study period, 2009, 2012, 2015 and 2017 were the years with the greatest ndT35, although the number differed among the three zones. For example, the ndT35 in 2012 was 20, 15 and 6 d respectively in RO, RA1 and RA2, while in the rest of indicated years the ndT35 was much higher in RO (between17 and 20 d) and in RA1 (between 10 and 16) than in RA2 (1 or 2 d). However, the highest average temperature during the growing period was recorded in 2017 followed by 2009, which were within the above commented years. In the other two years, the average temperature was slightly higher than the average but similar to that recorded in other years of the series analysed, like years 2011 or 2018, in which the number of days of warm extreme was smaller. It was also observed that, during the period of analysis, the number of days with warm extreme temperatures presented an increasing trend in RO and RA1, while it was not trend in RA2. The differences in the maximum growing season temperature between the warmest and the coolest year ranged between 3 °C in RA2 and in RO and 3.3 °C in RA1, while the differences between zones for that year in which the maximum was recorded, were 2.3 °C between RA1 and RA2 and 3.4 °C between RO and RA2.

Regarding precipitation, high variability existed among the years analysed. Annual precipitation ranged between 310 and 638 mm in RO and between 345 and 714 mm in RA. Years 2008, 2013 and 2018 were within the wettest years in the series, while 2009 was one of the driest of the analysed years and 2011, 2012 and 2017 were within the warmest years of the series analysed. However, precipitation during the growth season was quite scarce in some years, ranging between less than 100 and 456 mm in RA and between 122 and 353 mm in RO. In addition, the distribution of precipitation within the growing period varied among years. Water inputs during the growing season were smaller than crop evapotranspiration in most of years, giving rise to important water deficits, which were significantly high in all three zones in some years (e.g. 2011, 2012 or 2016 in which total water deficits up to 200 mm were recorded) and in other years in the warmest areas (e.g. 2019, 2010 or 2014 in which total water deficits of up to 175 mm were recorded) (data not shown).

Considering the average dates for budbreak and bloom found for these areas in previous studies and veraison, analysed in this research, it was observed that in all three analysed areas, the precipitation recorded during the periods between budbreak to bloom and veraison to maturity represented between 36 and 41 % of the growing season precipitation, on average. Precipitation recorded during the period between bloom and veraison represented between 25 and 33 % of the total growing season precipitation, while precipitation during the period veraison to maturity represented, on average between 11 and 20 % of the total growing season precipitation, which several years of null precipitation. Despite the variation among the three zones, the differences were not significant in the total amount of rainfall recorded in each period.

Vine response in the three zones under different climatic conditions. Differences in phenology among years and locations: Fig. S2 (Suppl. Fig. S2) shows the average dates for stage H (flowers separated) and M (veraison) in the three zones (RA1, RA2 and RO). Differences in phenology were observed among the study areas. The date for stage H ranged between 21st May to May 31st (average dates recorded in RO and RA2. The date for the stage H differed between both areas considered in RA, being up to 6 d earlier in RA1 than in RA2 and up to 10 d earlier in RO than in RA1. For veraison, the differences between zones were higher. The average dates ranged between August 3rd and August 17th, respectively in RO and in RA2. Average differences of about 7 d were observed between both zones of RA and of about 9 d between RA1 and RO (with differences of up to 18 d in some years). Regarding maturity, the threshold of PVAD= 13° was reached on average on Sep 13th (between Ago 27th and Sep 26th) in RO, while in RA that value was reached on average 13 d later (Sep 26th on average, ranging between Sep 6th and Oct 15th) in the zone RA1 and 21 d later (Oct 4th on average, ranging between Sep 13th and Oct 21st) in the zone RA2, which is located at higher elevation. The evolution of the PVAD along the ripening period also differed between zones (Fig. 1). The increase in the PVAD is faster in RO than in the two analysed zones of RA. The relationship between phenological dates (included the one for maturity PVAD= 13°) and temperature, showed that all three stages were mainly driven by TmaxGS, producing an advance of the phenological date with increasing temperatures.

Differences in grape composition among years and locations. Grape composition related to weather characteristics:

Due to the different climatic conditions recorded during the years of the period under study, differences in the grape composition during maturation as well as in the final characteristics were observed. The analysis of the influence of different temperature variables (Tmax, Tm, Tmin) on acidity and on the polyphenol contents, evaluated using a regression analysis, showed that TmaxGS was the variable that had higher influence. The relationship observed during the period under study (2008-2018) between total acidity and TmaxGS in the three areas showed a decrease of 0.33, 1.2 and 1.5 $g \cdot L^{-1}$, respectively in RO, RA1 and RA2 per 1 °C of increase in the maximum temperature during the growing season. Similarly, the malic acid concentration decreased between 0.43 $g \cdot L^{-1}$ in RA2 and 0.62 $g \cdot L^{-1}$ in RO per 1 °C of increase during the growing season. The number of days with Tmax > 35 °C showed in all plots a decrease of acidity when the number of days increased. Regarding polyphenols, the relationship between TPI and temperature showed an increase that ranged between 1.2 units in RA1 and 3.2 units in RO per 1 °C of increase in the TmaxGS. In addition, it was observed that colour may be also affected as colour intensity decreases with TmaxGS between 0.55 and 0.73 units per 1 °C increase in the TmaxGS. On the other

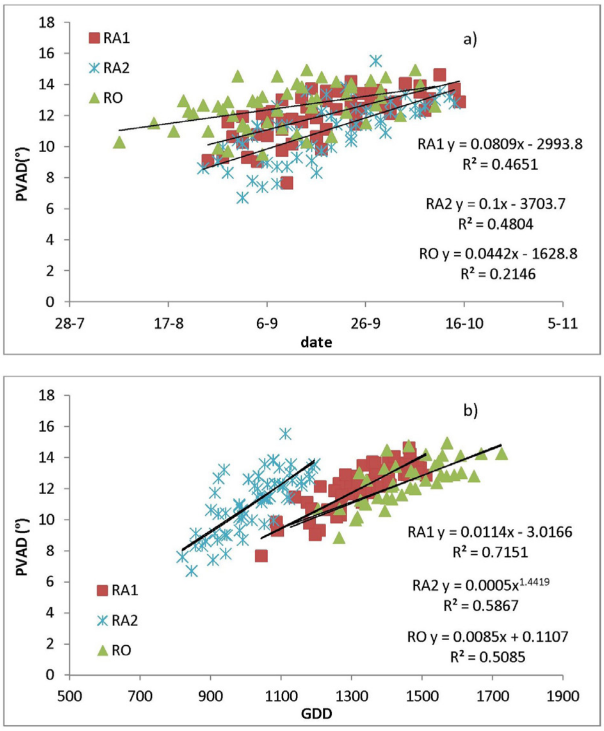

Fig. 1: Evolution of PVAD along the ripening period in the three analysed zones (RA1, RA2 and RO). **a**) evolution with time from veraison to maturity; **b**) relationship between PVAD and accumulated temperatures (GDD: accumulated mean tempertures -10 °C).

hand, it was observed that the anthocyanin concentrations decreased 36 and 20 g·L^{-1}, respectively in RA1 and RA2 for an increase of 1 °C in the TmaxGS. In RO, however, the best relationship was found with the accumulated GDD (base 10 °C, from 1st April), showing a decrease of concentration when the thermal accumulation reached at maturity was higher. A decrease of about 5 g·L^{-1} in the anthocyanin concentration for an increase of 10 GDD was observed. On the other hand, anthocyanin concentrations were also conditioned by water deficits. It was observed than in all three zones, anthocyanin concentration increased with water deficits, and in particular when those water deficits were recorded in the periods between bloom and veraison and between veraison and maturity. The increase was about 32.7, 18 and 13 g·L^{-1} respectively in RA2, RO and RA1, for 100 mm water deficit.

Differences in grape composition among zones: The threshold of 13° in the PVAD was reached and overpassed in both analysed plots of RO, in almost all years. The exception was the year 2018 in one plot, although the PVAD at harvest was near that value, and in the year 2008 in another plot. However, in Rioja Alta, there were several years in which the 13° threshold was not reached. In RA1, the PVAD was below 13° only in one plot in 2008 and it was near 13° in another plot in 2010, 2014 and 2016. However in RA2, the PVAD= 13° was not reached in 2013 in any of the analysed plots and it was below 13° in 2011, 2014 and 2018 in one plot, and in 2011 and 2018 in another plot. The evolution of the PVAD was driven by the accumulated GDD, and it was observed that its evolution was different in each analysed zone (Fig. 1). The threshold values were reached, even at lower temperature accumulation in the cooler area, although it took longer time periods.

Fig. 2 shows the relationship between anthocyanin concentrations and PVAD and the relationship between acidity and PVAD for the three analysed zones. The differences among the zones were greater for the acidity than for the anthocyanin concentration.

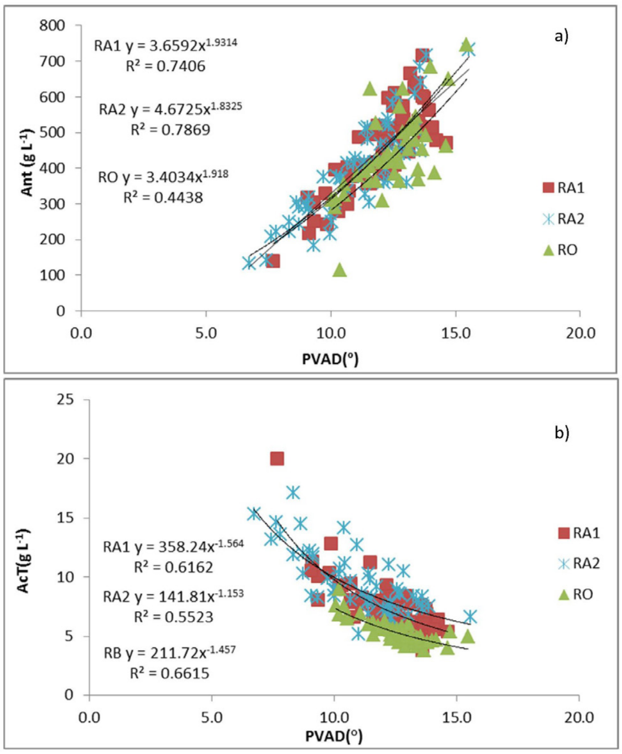

Fig. 2: Relationship between **a**) total anthocyanin and PVAD and **b**) total acidity and PVAD recorded in plots located in the three analysed zones.

The values of each grape parameter when the threshold for PVAD = 13° was reached (or the maximum PVAD obtained, which was between 12.5 and 13°) were compared in order to analyse the results of different years and locations. Fig. 3S (Suppl. Fig. 3S) shows the variation among years of the average values (acidity and phenolic compound variables) in the three analysed zones during the period under study. Regarding total acidity, the lowest acidity values throughout the period were observed in the plots of zone RO, and the highest values were observed in RA2. In all three zones, the highest values were recorded in the wettest years (2013, 2015, 2008 and 2018), while the minimum values were observed in 2012 and 2017, which were the warmest years of the series analysed (Suppl. Fig. 3Sa). Similarly, malic acid concentrations were higher in RA2 than in the other two zones with the lowest values in RO (Suppl. Fig. 3Sb). The differences between zones in most years were higher for malic acid than for total acidity.

For anthocyanin concentrations, the differences between zones were smaller than for acidity, reaching higher values in RA2 than in the other two zones (Suppl. Fig. 3Sc) in the driest and the warmest years (e.g. years 2009, 2012, 2017). The polyphenol index, however, was smaller in RA2 than in RA1 and RO in all years except in 2017 and the differences between zones did not follow a regular pattern (Suppl. Fig. 3Sd). Colour intensity did not follow a regular pattern in all years (Suppl. Fig. 3Se), although the trends were similar in the three zones in several years (e.g. 2012 or 2016). The differences from year to year in a given plot may be attributed to differences in the climatic characteristics. Nevertheless, the evolution of anthocyanin and acidity concentrations presented differences between zones during the ripening period. It was observed that, the final acidity values were always lower in the warmer than in the cooler areas (RO and RA, respectively), while the anthocyanin concentrations reached similar or even higher values in the cooler than in the warmer area (RA and RO, respectively) at maturity for a given PVAD (Suppl. Fig. 3S).

Discussion

During the period of analysis (2008-2018), a high variability in the weather conditions was recorded, both in temperature and in precipitation. Thus, the comparative analyses of the vine behaviour and grape evolution observed in those years may give us an idea of the potential of each area to maintain grape quality and production and can give an idea of the suitability of the areas located at different elevation under a climate change scenario.

The results observed during the period under study confirmed the differences in the thermal conditions among thel three studied zones, that made that the three zones were classified as different regions according to the Winkler index. The mean growing season temperature (TmGS) was 16.8 °C in RA1, 15.6 °C in RA2 and 18.6 °C in RO, which represent a wide range for 'Tempranillo' (Jones 2012), established between 15.5 °C and 18.5 °C. The differences in phenology observed among the analysed zones were in agreement with the climatic conditions and the delay in the zones located at higher elevation is in agreement with the later phenological dates recorded for the same variety at higher elevation (Consejo Regulador Ribera del Duero) and earlier dates recorded at lower elevation (DO Costers del Segre, at about 290 m a.s.l.) (unpubl. data). Those results indicated, for example, veraison ocurring between 25th and 31st July in vineyards planted at the lowest elevation (at about 290 m a. s. l.), while the same stage was reached between 15th and 20th Aug at the highest elevations (at elevation between 800 and 890 m a.s.l.).

The relationship between phenology and temperatures found in this research showed that TmaxGS and the number of warm extremes were the variables that had higher influence on the date at which a phenological event took place. This result agrees with the relationship between phenology and temperature found in other researches. Thus, Koufos *et al.* (2014) linked the earlier harvest to changes in maximum and minimum temperatures. Other authors have indicated the influence of maximum temperatures recorded in specific periods although some authors have indicated that mean and maximum temperatures are more important than minimum temperatures to describe the phenology timing (Ruml *et al.* 2015). In this respect, Bock *et al.* (2011) found that flowering was influenced by the maximum temperature of the preceding months (April to June) and veraison was dependent on temperature in later time periods (May to July). Ramos and Jones (2018) also indicated the relationship between bloom and veraison dates with Tmin and Tmax recorded when analysed 'Cabernet Sauvignon' in zones with different climatic characteristics.

Regarding maturity, the differences in the date at which it was reached in the three zones were consistent with the differences in the previous phenological dates. The threshold of PVAD= 13° considered in this research as usual value to determine maturity, was reached on average on Sep 13th in the warmer and lower area (RO), while that value was reached later in the cooler and higher areas (13 d later on (Sep 26 th) in the zone RA1, located at about 450 m a. s. l., and 21 d later (Oct 4 th) in the zone RA2 located at about 650 m a. s. l. That is to say, between Rioja Oriental and the cooler zone of Rioja Alta there is a difference of up to 21 d in the maturation of the grapes.

The advance in maturity was also driven by Tmax, being the effect higher in the cooler than in the warmer area (higher in RA2 than in RA1 or in RO, with an advance of 6.5, 10.0 and 8.0 d respectively in RA1, RA2 in relation to RO by 1 °C increase in TmaxGS). The effect of Tmin or Tm, however, was not significant in all zones. Following a similar approach to define maturity, Malheiro *et al.* (2013) confirmed the effect of Tmax recorded in some spring months on the date of harvest of different varieties cultivated in Portugal, and they also identified the influence of Tmin and Tm in some locations and varieties.

Thus, the differences in TmaxGS observed among the three zones, located at different elevation, during the period under study justify the earlier phenology in the warmer and lower zones (RO) in relation to the two other zones located at higher elevation, as TmaxGS in RO was up to 3.3 °C higher than in the RA zones. On the other hand, the advance in phenology with increasing temperatures agrees with results found in different viticultural areas around the world for different varieties (Duchêne and Schneider 2005, Sadras and Soar 2009, Bock *et al.* 2011, Webb *et al.* 2011, Webb *et al.* 2012, Ruml *et al.* 2015, among others), and can give an idea of expected changes under future change scenarios.

It is worth mentioning in this regard, that various management techniques have been proposed, in Rioja and with 'Tempranillo', for delaying grape ripening, such as late winter pruning (Zheng *et al.* 2017a), shoot trimming (Martínez de Toda *et al.* 2014, Zheng *et al.* 2017b) and minimal pruning (Zheng *et al.* 2017c). Each of these techniques allows delaying the ripening of the grape between 15 and 20 d. The application of any of these techniques to the vineyard of Rioja Oriental would allow delaying its ripening, making it to coincide with the Rioja Alta dates, that is, with cooler temperatures.

Regarding grape composition, in Fig. 3Sa (Suppl. Fig. 3 a) it is clearly observed that the total acidity was higher in the cooler areas and located at higher elevation (RA1 and RA2 zones) than in the warmer areas located at lower elevation (RO zone) and that the acidity decreases as PVDA increases. Similarly, the malic acid concentrations were higher in RA2 than in the other two zones with the lowest values in RO (Suppl. Fig. 3Sb), although the differences between zones in most years were higher for malic acid than for total acidity. The decrease in acidity during ripening is mainly due to the evolution of malic acid, which decreases during respiration and the optimum temperature for its combustion is about 30 °C. In warmer areas, like RO zone, there is greater degradation of acids and greater accumulation of sugars, which accentuates the imbalance of the wines and the heavy sensations due to the higher alcohol content.

The recorded values of acidity are consistent with the differences in temperature recorded between zones and with the differences observed within a given zone between warmer and cooler years. These results agree with those of other authors, who indicate decreasing acidity in berries with increasing temperatures (Coombe 1987, Spayd *et al.* 2002, Torres *et al.* 2017) and that grape berries from a hot environment are likely to have lower acidity than berries from a cool environment (Sadras and Moran 2013). The results are also in agreement with those observed at higher elevation by Ramos *et al.* 2015b for this variety. Due to the low acidity, characteristic of the 'Tempranillo' variety, its cultivation is more suitable in the upper than in the lower areas (Rioja Alta *vs*. Rioja Oriental, in the study case), where the wines preserve a higher level of acidity (Balda and Martínez de Toda 2017).

Regarding the anthocyanin concentrations, a decrease of concentration with increasing temperatures was observed, which has been also indicated by other authors. In this respect, De Rosas *et al.* (2017) indicated that day high temperatures reduced total anthocyanins. Similarly, Shinomiya *et al.* (2015), Fernandes de Oliveira *et al.* (2015) and Zarrouk *et al.* (2016) found that high temperatures decreased anthocyanin contents. It was also observed that the relationship between anthocyanins and PVAD is a bit higher in the cooler areas (RA1 and RA2) than in the warmer areas (RO) (Fig. 2), maintaining that difference throughout the ripening. That is, to obtain the same level of anthocyanins, a greater PVAD was needed in the warmer and lower area (RO) than in the higher and cooler areas (RA1 and RA2). This is what is known as thermal decoupling between anthocyanins and sugars and is that high temperatures delay the synthesis of anthocyanins in relation to the evolution of PVAD (Sadras and Moran 2012). It is confirmed that this decoupling exists in the warmer zone of Rioja Oriental when compared to the cooler zone of Rioja Alta and that the increase in temperature favours the high probable degree, delaying the synthesis of anthocyanins, so that the wines produced in warmer zones tend to higher alcohol contents and less intensity of colour.

Water deficit is another important factor, which may have an opposite effect than temperature on the final grape composition, as it can affect the metabolic pathways of some compounds (Deluc *et al.* 2009). Water deficits may increase anthocyanins (Hochberg *et al.* 2015) and total phenols (Cáceres-Mella *et al.* 2017), although the different compounds may be affected in different ways depending on the cultivars and rootstocks (Berdeja *et al.* 2015, Hochberg *et al.* 2015). Castellarin *et al.* (2007) indicated that water deficits consistently promote higher concentrations of anthocyanins in red winegrapes and their wines, although the effect may be different depending on when this deficit occurs. In this research, the anthocyanin concentrations were affected by both, temperature and water deficit, decreasing with increasing temperature accumulation during ripening and increasing when water deficit increased in the period between bloom and veraison. Thus, temperature and water deficits can have additive effects, as mentioned by Bonada *et al.* (2015). The effect of water deficits seemed to be higher in RA2, but it was the area where lower deficits were recorded.

This is an important issue regarding decoupling that may appear between anthocyanin and sugar content in the berries, which was already mentioned before in relation to temperature effect, in which water deficit seems to be implicated. For the PVAD threshold established to define maturity in this research, it was observed that for a given temperature recorded, the level of the water deficit influences the ratio anthocyanin/PVAD. For example, comparing in RO, years 2009, 2011, 2015 and 2017, all of them with high temperatures, it was observed that ratios in 2015 and 2017 were much lower than in 2009 an in 2011. The high temperature recorded in those years could justify the low ratios in 2005 and 2017, but not the higher ratios observed in 2009 and even higher in 2011. The main difference among the years were the water deficits recorded during the growing season, which were higher in 2011 than in the other two mentioned years. The ratio anthocyanin/PVAD was higher in the cooler and higher areas (RA1 and RA2), than in the warmer and lower areas (RO) in the years with lower water deficits and opposite in the year with the highest deficit. However, water deficit in RA2 was much lower than in the other two zones.

Conclusion

The spatial and temporal variability existing within the Rioja DOCa, allows extracting useful information about the potentiality of areas located at different altitude and with different climatic conditions to produce high quality grapes of the 'Tempranillo'. High differences in all phenological stages exist among zones whit different altitudes, which could be of up to 16 d for veraison and up to 27 d for maturity between zones that differ in about 300 m in elevation. Differences in the evolution of grape composition during ripening and in the final values were also observed among years and elevations due to the differences in the climatic conditions. It was confirmed the effect of high temperatures on acidity and on anthocyanin concentration and also the effect of water deficits recorded along the growth period, which may be additive. The differences found in this research among the higher and the lower elevated areas, support the idea of exploring cooler areas and reinforcing viticulture at higher elevation, which could give more favourable conditions and less imbalanced grape composition than warmer conditions.

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