Relationships between grape composition of Tempranillo variety and available soil 1 2 water and water stress under different weather conditions 3 4 María Concepción Ramos^{1*}, Eva Pilar Pérez-Álvarez², Fernando Peregrina³, 5 Fernando Martínez de Toda⁴ 6 ^{1*}Department of Environment and Soil Sciences. University of Lleida - Agrotecnio. 7 Alcalde Rovira Roure, 191, 25198, Spain. cramos@macs.udl.es 8 ²Centro de Edafología y Biología Aplicada del Segura (CEBAS), CSIC, Campus 9 10 Universitario de Espinardo, Ed. 25. E-30100, Espinardo. Murcia. epperez@cebas.csic.es 11 ³INEA-Escuela Universitaria de Ingeniería Agrícola. Universidad Pontificia Comillas. Cno. Viejo de Simancas, km 4,5 - 47008 Valladolid. fperegrina@comillas.edu 12 13 ⁴ICVV (Universidad de La Rioja, CSIC, Gobierno de La Rioja), c/ Madre de Dios, 51, 26006 Logroño, Spain. fernando.martinezdetoda@unirioja.es 14 15 *corresponding author: MC Ramos, cramos@macs.udl.es, University of Lleida -16 Agrotecnio. Alcalde Rovira Roure, 191, 25198, Spain. 17

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Con formato: Español (alfab. internacional)

19 Relationships between grape composition of Tempranillo variety and available soil

20 water and water stress

21

22 Abstract

23 The aim of the research was to analyse the relationship between grape composition of 24 the Tempranillo variety, cultivated under rainfed conditions, and available soil water along the growing cycle. The study was conducted in the Rioja DOCa (Qualified 25 26 Designation of Origin (Spain). Grape composition (berry weight, acidity and phenolic 27 composition) between veraison and maturity was analysed in non-irrigated vines and related to available soil water along the growing cycle, simulated for the period 2008-28 2018, taking into account soil properties and the weather conditions recorded at each 29 30 location. Soil water was simulated for each plot and year analysed, after calibration in 31 one plot and the available soil water (ASW) was evaluated under the different conditions. The results showed that soil properties conditioned available soil water, 32 which influenced berry weight, acidity, anthocyanins and total polyphenol index. Under 33 34 the rainfed conditions in which the vines were cultivated, vines suffered from moderate to high water stress in some periods along the growing cycle. The results showed the 35 most critical periods regarding water availability for grape composition. An increase in 36 37 available soil water four and seven weeks after the stage H and at the end of the ripening period increased acidity and berry weight and decreased pH, since bloom until 38 harvest increases berry weight and acidity, while for anthocyanins and other phenolic 39 40 compounds an increase in available soil water after just before veraison and in particular 41 during ripening.veraison decreases their concentrations.

- **Keywords**: acidity; anthocyanins; berry weight; polyphenols; soil characteristics;
- 44 available water content.

1 1. Introduction

Berry composition is influenced by factors such as soil characteristics, management, 2 3 cultivar and climate, (van Leeuwen, 2004). Among all these factors, climate plays an 4 very important role on berry composition (Jones and Storchmann, 2001; Robinson et al., 2012). The vine cultivars that can be grown in a given location are conditioned by 5 temperature (Jones et al., 2012) and its variations affect grape development during the 6 7 growing period and its final composition (Sadras and Moran, 2013; Webb et al., 2012; 8 Greer and Weedom, 2013; Ovadia, et al., 2013). However, within a specific climate zone, soil characteristics are important factors controlling vine development and grape 9 composition (Cheng et al., 2014; Zerihun et al., 2015). Soil physical properties, and in 10 11 particular soil particle distribution and porosity govern the volume of soil that can be explored by roots, and also affect water and nutrient movement and soil water storage 12 (Lanyon et al., 2004; Seguin, 1986; Costantini et al., 2006; Ramos et al., 2015), as well 13 as water availability and finally the vine water status (Costantini et al., 2010; 14 15 Tramontini et al., 2013). Thus, water availability could play an important role on vine 16 development and the final production, and it could be used to determine vine water 17 stress.

Vines are sensitive to water stress during the growing cycle and both early and lateseason deficits decreased yield (Matthews et al., 1987). Berry growth is more sensitive to water deficits after bloom and if the dry periods take place after fruit formation they lead to a decrease in the water used, which has also an impact in grape quality. However, reductions in berry growth caused by water stress during the budbreak-bloom period cannot be reversed by supplemental water inputs during the bloom-veraison and veraison-harvest periods (Grimes and Williams, 1990). These authors indicated that water used by plants decreases when soil water is depleted to the point that plant is stressed, and yields also will be reduced. Peacook (2005) indicates that a water deficit up to 50% of evapotranspiration (ETc) has a minimum effect on yield. However, yield decreases if this threshold is overpassed. In this respect, Ramos and Martínez-Casasnovas (2010) confirmed that the years in which water deficit during the growing period was higher than 50% ETc, were years of low yield, and that yield was affected by water available during the budbreak-bloom period.

In addition, water deficits can affect also berry grape-composition (Esteban et al. 2001; 32 Deloire et al., 2005; Downey et al. 2006; Gómez-Míguez et al., 2007; van Leeuwen et 33 34 al., 2009), being the effect dependent on the level of stress and the time period when it 35 appears (Ojeda et al., 2002; Roby et al., 2004; Castellarin et al., 2007). Intrigliolo et al. 36 (2012) found that late water deficits affect berry sugar accumulation due to the effect of 37 water stress on leaf photosynthesis. Cooley et al. (2017) found an increase in total acidity under different irrigations treatments and that the malic acid concentration of 38 39 grape juice was reduced under both regulated and prolonged deficit irrigation, resulting in an enhancement of the tartaric/malic acid ratio. Bellvert et al. (2016) also indicated 40 that pre-veraison water stress negatively affected aroma quality, titratable acidity, and 41 42 malic acid.

Regarding the effect on anthocyanins and other phenolic compounds, it has been reported that water deficit changed anthocyanin composition, as well as the composition and the accumulation of flavonols or proanthocyanidins (Torres et al., 2018). In general, water deficit enhanced the accumulation of phenylpropanoids, monoterpenes, and tocopherols, while for other compounds such as carotenoids and flavonoids, the accumulation depend on the grapevine development state (Savoi et al., 2016) and on the stress level. In this respect, Balint and Reynolds (2014) found that total anthocyanin 50 concentration was highly affected by irrigation treatments, finding the lowest 51 anthocyanin and phenol concentrations under the irrigation treatment corresponding to 52 the 100% of the crop evapotranspiration (ETc). Similarly, Cooley et al. (2017) found wine colour density, anthocyanin, ionised anthocyanin and phenolic substances being 53 affected by the irrigation treatment. Buchetti et al. (2011) also indicated that water 54 55 deficits consistently increased anthocyanin concentration by increasing content per berry and reducing fruit growth. Talaverano et al. (2017), Mendez-Castabel et al. (2014) 56 57 and Ou et al. (2010), among others, indicated the influence of water stress in some alcohols and on different volatile compounds responsible of aroma characteristics, 58 59 which may have consequences on wine sensory perception.

The research was carried out in the Rioja DOCa, which is the most important producer 60 region of the Tempranillo (Vitis vinifera) variety worldwide. In previous research 61 62 carried out in the study area, the effect of weather conditions and the influence of some soil properties in the response of the vine were already analysed (Ramos and Martínez 63 64 de Toda, 2019). In this research, the aim was to acquire a deep knowledge of the influence of available soil water and the time in which water stress can me-be more 65 critical for grape composition. To that end, grape composition of the variety 66 Tempranillo, cultivated under rainfed conditions, was analysed in years with different 67 68 weather characteristics, and related to the stress conditions that the vine suffered along 69 its growing cycle. This variety is adapted to Mediterranean climate and it has been s is 70 now being expanded to other counties (among them, Australia Chile, Greece, USA and South Africa). Thus, the results observed in this specific area related to the influence of 71 72 available water and water stress on grape quality could be extrapolated to other areas 73 around the world, taken into account the soil characteristic where the vines are planted.

74

75 2. Material and methods

76 The research was carried out in the DOCa Rioja (Spain) grapevine growing area. This region is located in North-central part of northern Spain and has covers-about 64000 ha 77 of vines, mostly red varieties. The vines are cultivated from the terraces of the Ebro 78 River to elevations up to about 700 m a.s.l. The climatic conditions recorded along the 79 regions allowed stablishing different zones with Atlantic and Mediterranean influence, 80 which are named, respectively, Rioja Alta (about 26780 ha with Atlantic influence), 81 Rioja Oriental (about 23870 ha, with Mediterranean influence) and Rioja Alavesa 82 (about 13000 ha), with intermediate climatic influence (Consejo Regulador DOCa 83 2017). Tempranillo is the main cultivated variety in the region, which represent about 84 80% of the vineyard area. Most vineyards of the Rioja DOCa use tillage for soil-surface 85 86 management, and the norm of this appellation limits the red grape yield to 6,500 kg/ha.

87 2.1. Study area

88 This research included the analysis on six plots located at similar elevation (between 428 and 465m a.s.l.) in the municipalities of Haro (P1 and P2), Cenicero (P3), 89 Fuenmayor (P4), San Vicente de la Sonsierra (P5) and San Asensio (P6) in Rioja Alta 90 91 (Fig. 1). All plots were planted with the Tempranillo variety, between 1987 and 2000 (1993, 1997, 1998, 2000, 1987, 1990 and 1999, respectively); they were bush trained in 92 93 double cordon, in a patter of 2.5-2.7m between rows and 1.2-1.3m between plants, which means 2800 to 3,500 vines/ha. The vines were and cultivated under rainfed 94 conditions. , with 3,000 to 3,500 vines/ha. Rootstock and clone???? 95

An additional plot was considered (P7), in which soil water was measured during four
years. This plot was situated in the municipality of Nájera (Fig. 1), and planted with
similar pattern (2.7 m between rows and 1.3 m between plants).

Comentario [U1]: He intentado añadid algo más de lo que el segundo revisor pide, aunque no creo que se lo vuelvan a enviar De la parcela donde se medió la humedad si que se sabe e portainjerto y el clon, ¿ tienes alguna idea del portainjjerto que puede haber en las otras parcelas?

Comentario [U2]: clone RJ.26 on Richter 110 rootstock.

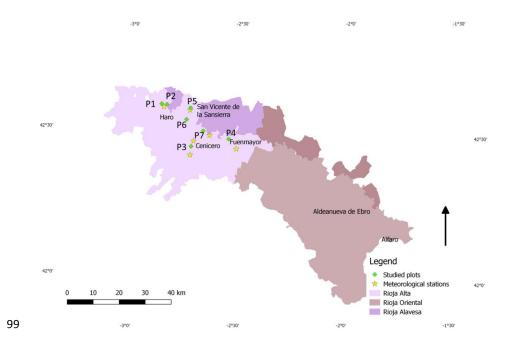


Fig. 1. Map of the three zones in which are divided the Rioja DOCa growing region
(Rioja Alta, Rioja Oriental and Rioja Alavesa). Location of the seven plots (P1 to P7)
and the six meteorological stations used in this research.

104 2.2. Climate data

105 The weather conditions recorded during the studied period (2008-2018) were analysed using the information from the meteorological stations of Haro, Uruñuela, Logroño, 106 107 Nájera and San Vicente de la Sonsierra, which were the nearest stations to the analysed 108 plots (Fig. 1). Those stations belong to La Rioja Government. Daily maximum and 109 minimum temperatures, precipitation, solar radiation, relative humidity and wind speed 110 were analysed. Crop evapotranspiration was also evaluated for each meteorological 111 station, which was calculated from the potential evapotranspiration obtained using the 112 FAO Penman Monteith equation and the crop coefficients proposed by Allen et al. (1998). For the plots located between two observatories a weighted average was 113

estimated taking into account the inverse of the distance to each station, and when itwas necessary a correction for differences in elevation was applied.

116

117 2.3. Soil properties

118 The soils of the selected plots are classified as Calcixerollic Xerochrept (IGN 2006). 119 Soil characteristics of the studied plots related to soil organic carbon, soil particle 120 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 121 122 (ESDAC; esdac.jrc.ec.europa.eu, European Commission, Joint Research Centre). The 123 soils of the studied plots have clay contents that range between 19.7 and 25.5%; silt 124 contents that range between 34.6 and 45.3% and sand contents that ranged between 29.2 125 and 39.3%. The organic carbon content varied between 0.49 and 1.53%. The water 126 retention corresponding to field capacity ranged between 25.4 and 30.1% and that for the wilting point ranged between 10.9 and 15.3%. The soils characteristics of the plots 127 used in this research are shown in Table 1. 128

Plot	Elev	Clay	Silt	Sand	Coarse	OM	FC	WP
	(m a.s.l)	(%)	(%)	(%)	elements	(%)	(%)	(%)
					(%)			
P1	438	22.8	34.6	42.6	13.2	1.1	26.6	12.8
P2	465	22.3	38.4	39.3	14.2	1.00	26.9	12.4
P3	450	25.5	45.3	29.2	13.0	0.75	30.1	13.7
P4	428	19.7	41.7	38.6	18.0	1.53	25.4	11.0
P5	440	22.2	43.2	33.9	17.8	1.40	27.6	15.3
P6	457	25.9	43.4	30.7	14.5	0.49	27.0	10.9
P7	450	18.5	43.2	38.3	12.0	0.96	27.0	15.5

Table 1. Soil properties of the analysed plots (P1 –P7). (Elev: plot altitude in m above
sea level; OM: organic matter; FC: field capacity and WP: wilting point).

133

134 2.4. Available soil water

135 In order to evaluate the effect of available soil water on grape composition, soil water was simulated for each plot and year, considering soil properties and the weather 136 137 conditions recorded in each year. The Vineyard-Soil Irrigation Model (VSIM -138 https://sites.google.com/a/csumb.edu/vsim/) model was used to simulate soil water. This model allows simulating soil water content at daily time scale for the whole 139 140 profile, based on a soil water balance, which included water inputs (rainfall and 141 irrigation in case the crop was irrigated), crop evapotranspiration and drainage. The inputs for the model included weather (daily temperature, precipitation and potential 142 143 evapotranspiration) and soil (soil particle distribution and gravel contents, water

retention capacity at -33 kPa and 1500 kPa and rooting depth) characteristics. Regarding
the vines, the model takes into account vine and row spacing and cover crop.

The model was calibrated and validated with the data recorded in P7. Soil water content 146 147 was monitored in that plot from 2009 to 2012 at different soil depths (30, 60 and 100 cm), using TDT (Time Domain Transmissometry) GroGraph Moisture Solution probes 148 149 (ESI Environmental Sensors Inc, Sidney BC, Canada), which recorded information at 150 30 min intervals. These data were divided into two series: the periods 2009-2010 and 2011-2012 were used for model calibration and validation, respectively. The model 151 152 performance for both calibration and validation was analysed using three statistical 153 methods: the ratio of the root mean square error to the standard deviation (RSR); the Nash-Sutcliffe efficiency (NSE; Nash and Sutcliffe 1970) and the percent bias (PBIAS, 154 155 %; Gupta et al. 1999). (Equations.1, 2 and 3, respectively).

156
$$RSR = \frac{\sqrt{\sum_{i=1}^{n} (Y_m - Y_s)^2}}{\sqrt{\sum_{i=1}^{n} (Y_m - \overline{Y})^2}}$$
(1)

157
$$NSE = 1 - \frac{\sum_{i=1}^{n} (Y_m - Y_s)^2}{\sum_{i=1}^{n} (Y_m - \overline{Y})^2}$$
 (2)

158
$$PBIAS = \frac{\sum_{i=1}^{n} (Y_m - Y_s) * 100}{\sum_{i=1}^{n} (Y_m)}$$
 (3)

159 Where Ym is the measured value, Ys is the simulated value and \overline{Y} is the mean of the 160 measured values of the parameter analysed.

161

162 Then, the model was run for each plot and year. Taking into account the maximum soil
163 water content at field capacity (FC) and the wilting point (WP), the maximum available
164 soil water (water held o<u>f m</u> the soil between its field capacity and permanent wilting

165 point) of each soil was calculated (AWC). The available soil water (differences between soil water and the wilting point) (ASW) was evaluated along the growing cycle for each 166 167 plot and year. Particular attention was paid to the periods between flowers separated and 168 veraison and after veraison. The information about phenology, according to the Baggliolini scale, was obtained from the Consejo Regulador of Rioja DOCa (personal 169 170 communication). The water stress conditions that the existing ASW implied for the vine 171 were also analysed. Vine water stress was quantified considering the fraction of the 172 ASW, estimated considering the ASW and the AWC. The levels of water deficit (weak, moderate and severe) were stablished from the results observed by Pellegrino et al. 173 174 (2005) and van Leeuwen et al. (2009). The thresholds were stablished in terms of the 175 percentage of the AWC as follow: weak water deficit: 32-20% of AWC; weak to 176 moderate water deficit: 20-8% of AWC; moderate to sever 8-2% of AWC and severe water deficit for values < 2% of AWC. Those thresholds were considered to estimate 177 178 the vine water deficit.

179

180 **2.5. Grape composition**

Grape composition was analysed weekly during ripening (from veraison to maturity) for 181 the period of analysis (2008-2018). Berry weight (weight of 100 berries-WB), sugar 182 183 content (expressed as probable volumetric alcoholic degree-PVAD), total acidity (AcT), malic acid (AcM), pH, total anthocyanins (AntT), total polyphenol index (TPI) and 184 colour intensity (CI) were evaluated for each plot and year. The information was 185 186 provided by the Consejo Regulador of Rioja DOCa and it was obtained following the 187 methods recommended by the OIV (OIV, 2012). The differences between plots were 188 evaluated taking into account the soil water content and the ASW in each plot. A partial Least Squares (PLS) regression between grape parameters and the ASW was performed 189

190 in order to analyse its influence and the timing when it occurred. Weekly values of 191 ASW from the stage H until ripening (defined when $PVAD = 13^{\circ}$ was reached) were 192 included in the analysis. The data corresponding to two plots with different 193 characteristics (P2 and P6) regarding the AWC were used to create the model while the rest of plots were used in the cross validation. The grape parameters were analysed by 194 195 groups: acidity (AcT, AcM and pH), anthocyanin and phenolic characteristics (AntT, TPI and CI and the ratio AntT/PVAD) and BW, considering the values of these 196 197 variables when the PVAD reached 13°.

198 **3. Results**

199 3.1 Weather conditions recorded during the period of study

200 Years with different characteristics were recorded during the analysed period. The 201 average maximum, minimum and mean temperature (TmaxGS, TminSG and TmGS) and the precipitation (PGS) recorded during the growing season (GS: April-October) in 202 203 the observatories included in this research are shown in Table 2. The mean growing season temperature (TmGS) ranged between 16.9 and 17.6°C. The maximum growing 204 season temperature (TmaxGS) varied between 23.8 and 24.5°C, while minimum 205 206 growing season temperature the TminGS) ranged between 10.6 and 12.5°C. The 207 differences among meteorological stations were slightly higher for TminGS_than for TmaxGS. The average PGS growing season precipitation ranged between 179 and 257 208 209 mm (Table 2), but with high variability from year to year. Water inputs during the 210 growing season were smaller than crop evapotranspiration in most of years, giving rise 211 to important water deficits, which were not covered by the water reserves accumulated in the soil during the dormant period. Within the analysed period, years 2008, 2013 and 212 213 2018 were within the wettest years in the series, while 2009, 2011 were the driest. In

214	addition, both mentioned years were within the warmest years of the series analysed,
215	with 2017 as the warmest one. In year 2016, despite annual precipitation was above the
216	average, precipitation recorded in the growing season was very low, even lower than in
217	the mentioned dry years. Year 2014 presented intermediate conditions regarding both
218	temperature and precipitation, which was more homogenously distributed along the
219	year, with about 50% of annual precipitation recorded during the growing season.
220	According to the weather conditions observed, some years were selected to analyse the
221	vine response: 2009, 2011 and 2016 as dry years; 2008, 2013 and 2018 as wet years;
222	2017 as a warm year and 2014 as a year with intermediate conditions.

Table 2. Average mean, maximum and minimum temperature (GS-TmGS, GS-TmaxGS, GS-TminGS), precipitation (GSPGS) and crop evapotranspiration (GS-ETcGS) recorded during the growing season (April-October) and precipitation recorded in the hydrological year (1st - Oct- 30th Sept) (PHY) in the studied period (2008-2018), in the meteorological stations used in this research.

Meteorologica	GS	GSTmaxG	GSTminG	PHY	GS	GS
l station	Tm <u>GS</u> (°C)	<u>§</u> (°C)	<u>§</u> (°C)	1 st Oct- 30 th Sep	P <u>GS</u> (mm)	ETc <u>GS</u> (mm)
				(mm)		
Logroño	17.6±0.	24.1±1.0	12.1±0.5	472±12	241±10	495 ±42
	8			2	9	
Uruñuela	16.9±0.	24.4±1.1	10.6±0.6	477±10	246±91	403±10
	8			8		0
Haro	17.4±0.	24.4±1.0	12.1±0.5	464±11	190±93	426±26
	7			3		
Nájera	17.5±0.	24.5±1.2	11.6±0.5	369±10	179±58	437±26
	9			5		
San Vicente	16.9±0.	23.8±0.9	10.9±0.5	573±12	257±84	488±43
de la	6			1		
Sonsierra						

228

230 **3.2. Differences in phenology among years**

The average phenological dates or the analysed years were 26th May±8 days for stage H, 12th August±7 days for stage M (veraison) and ripening was reached on average on 30th September±11 days. In the wet years, the dates of stages H and M were on average 10 days later than in the dry years, while ripening took place up to 18 days later. In the warmest year, veraison and ripening occurred 8 and 9 days earlier, respectively, than in the coolest years, respectively.

237 3.3. Soil water measured and simulated under different climatic conditions

238 3.3.1 Model calibration.

Figure 2 shows the measured and simulated soil water in the profile for P7, for the 239 calibration (2009 and 2010) and validation periods (2011 and 2012). Despite the 240 241 different characteristics of the years, the model was good fitted. Validation is presented separately for both years. The statistics are shown in Fig.2. For the calibration period, 242 243 PBIAS was about 5%, the NSE was 0.825 and the RMSE was 0.42, which could be 244 considered as very good (according to the criteria proposed by Moriasi et al. 2007). For 245 the two years used in the model validation, the PBIAS were 0.75 and -1.12%, the NSE were 0.66 and 0.78, and the RSR were 0.48 and 0.58, respectively. This means 246 247 satisfactory results (between good and very good).

- 248
- 249

250 <u>3.3.2.</u> Simulated soil water contents and available soil water for the selected plots 251 and years.

Using the model, soil water was simulated for each selected plot and year. Due to soil 252 characteristics, the maximum AWC that each soil can accumulate in the profile varied 253 254 between 139.5 mm in P1 and 165.1 mm in P3. In the rest of the plots, the values were 255 145.6, 144.5, 155.6 and 158.7 mm, respectively for P2, P4, P5 and P6. The ASW along 256 the growing cycle in each plot in the analysed years is shown in Fig. 3. It can be observed that, the ASW reached the maximum capacity in almost all plots and years at 257 the beginning of the growing cycle. However, after stage H, the ASW decreased very 258 fast reaching the 50% of the maximum holding capacity after few weeks, although it 259 varied among plots and years. 260

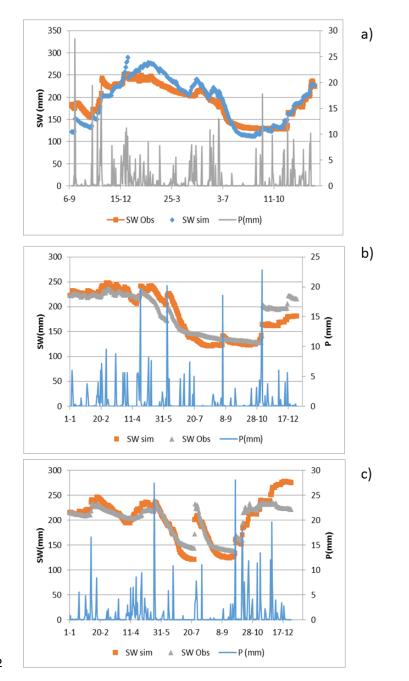


Fig. 2. Simulated (SW sim) and measured (SW Obs) soil water in P7 during a) the calibration period (2009-2010) and b) and c) validation period (2011 and 2012).

In dry years, like 2009, the 50% of the maximum ASW was reached after two weeks 265 from stage H in P6 and after five weeks in P2 or P3 (Fig. 3). In addition, in the 266 267 following weeks, there were differences between these two plots, not only in the 268 minimum level of water reached but when it took place. While in P3, ASW was decreasing, reaching the level below 20% of the AWC (considered as the threshold to 269 270 define moderate to weak water deficit) after eight weeks, in the case of P2 it happened after six weeks, with the minimum level reached at veraison, which was about 7% of the 271 272 maximum capacity. In that situation vine stress was much higher in P2 than in P3. In other plots, intermediate situations were given. For example, in P6, with an AWC 273 274 relatively high, the ASW reached the value corresponding to 50% of the maximum two 275 weeks after stage H, but the decrease was more moderate and the minimum values were 276 higher than in P2 (Fig. 3). The differences among plots in other dry years were quite 277 similar. In 2011, for example, the value corresponded to the 50% of the maximum ASW was reached after 2 and 5 weeks after stage H in P3 and P2, respectively, while the 278 279 value corresponding to 20% of the AWC was reached after 5 and 7 weeks, respectively, and the minimum values reached in P3 was near double than in P2. In 2016, despite 280 281 being the annual precipitation higher than the average in the area, the precipitation recorded during the growing season was scarce and vines started to suffer stress two or 282 three weeks after stage H (Fig. 3). 283

For wet years like 2013, ASW was higher that 50% of the maximum capacity during up to nine weeks after the stage H in P3 and P6, and about during up five or six weeks in the plots with lower AWC (like P1 and P2). The ASW was above 20% until one week before veraison in P3, and about 5 weeks before veraison in the plots with lower AWC (Fig. 3). In years with high temperatures, like 2011 and in particular in 2017, the higher evapotranspiration made that soil water decreased earlier than in the above mentioned years and the 50% of the AWC was reached just one or two weeks after stage H, depending on the plots. Although the minimum values of ASW reached in all plots were higher than in drier years, water deficits although weak (according to the stablished thresholds) were maintained during longer time, between six and eight weeks before veraison, which could also have negative effects for grape development.

At veraison, ASW usually reached very low values, around 10% of the maximum capacity, except in the very wet years (e.g. year 2013) (Fig. 3). However, even in the driest years (e.g. 2009 or 2011), those minimum values were reached before in the plots with lower AWC.

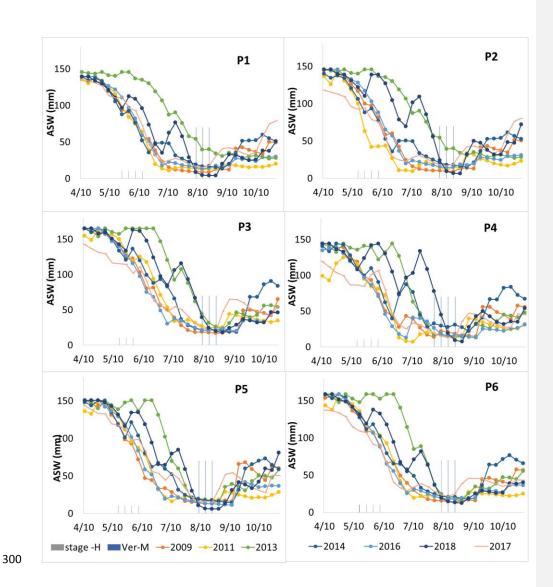


Fig. 3. ASW along the growing cycle in the selected plots (P1-P6), in years with different weather conditions (2009, 2011 and 2016: dry years; 2013 and 2018: wet years; 2017: very high temperatures; and 2014: intermediate characteristics). The periods in which the stage H (stage -H) and veraison (Ver-M) took place are also indicated.

306 3.4. Differences in grape composition among plots and years

307 Due to the different climatic conditions, recorded during the years of the analysed 308 period, differences in the grape composition during maturation were observed, both in 309 timing and in the final characteristics. Maturity was defined in relation to a given probable alcoholic degree (PVAD). Thus, the grape characteristics were analysed during 310 311 ripening (from veraison to harvest) and in particular when the PVAD equal to 13° were reached, in order to compare the differences among plots. Figures 4-6 show the 312 313 variation among years of the berry weight, acidity and phenolic compounds in the analysed years, respectively. In these figures it is also shown the average water content 314 315 between budbreak (stage C) and stage H and between stage H and veraison (stage M) recorded in each year. 316

Regarding berry weight, although it was clear that in all plots the lowest values were recorded in 2011 (one of the driest and warmest years) and the highest values in 2018 (one of the wettest year), the differences among dry and wet years were not clear when all analysed years were evaluated as a whole (Fig. 4). Thus, water content may be not the main or the only factor that affect berry weight, or it should be also considered the time when the highest and the lowest water levels were recorded.

323 Total acidity was, in general, higher in the wet than in the dry years, with some 324 differences between plots (Fig, 5). In wet years, the lowest value was recorded in P3, 325 while in dry years it was recorded in P4. However, in the warmest year analysed (2017), the differences among plots followed the same pattern than in the wet years until 326 reaching a PVAG=12°, but the lowest values were recorded in P1 and P2 when the 327 $PVAD = 13^{\circ}$ was reached. In that year, ASW between stage H and veraison (stage M) 328 329 was higher in P3, followed by P5, in which total acidity recorded the highest values at the end of ripening (Fig. 5). Similarly, for the malic acid, the lowest values were also 330

recorded in P3, however, there were not a clear pattern among the rest of plots regarding water available (Fig. 5). Thus, water available seems to affect total acidity but it is necessary to investigate the time in which this water is available to confirm in what stage water can have higher effect. In addition, the effect of temperature on acidity was also confirmed. The lowest acidity values at ripening were observed in the warmest year (2017).

337 For the total anthocyanin concentrations, the highest values were observed in the dry 338 years like 2009 or 2011. However, in 2016, which was also a dry year during the 339 growing season, total anthocyanin concentrations were lower than in the other dry years 340 (Fig. 6). As it was already said, 2016 recorded higher annual precipitation than the average, but precipitation recorded during the growing season was very low and ASW 341 342 was lower during the whole growing season than in other years, even in the first stages. 343 This was one of the differences between that year and 2009 and 2011. Another 344 difference was the temperature, which was lower in 2016 than in the other two 345 mentioned years. In 2017, which was the warmest year of the analysed series, the 346 differences in total anthocyanin levels among plots were lower. Nevertheless, it was 347 confirmed that P3 recorded always the lowest levels, being the plot in which ASW during the period between stage H and veraison was the highest (Fig. 6). Thus, it is 348 necessary to take into account not only water available in the whole growing season but 349 350 in different stages.

The TPI index was smaller in the wet than in the dry years, but similarly to that commented for the anthocyanin concentrations, the TPI showed lower values in 2016 than in 2009 and 2011. The highest differences among plots were found in years in which there were differences in ASW not only during ripening but also during the previous stages (Fig. 6).

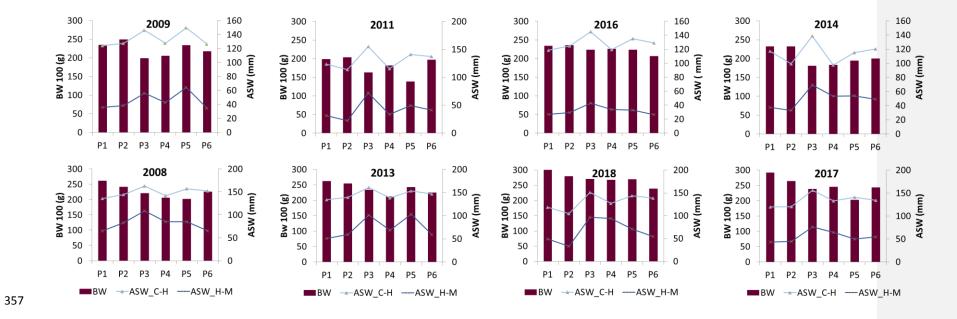


Fig. 4 Average values of the 100-berry weight (BW-13) recorded in the analysed years (2009, 2011 and 2016: dry years; 2014: intermediate

- characteristics; 2008, 2013 and 2018: wet years; 2017: very high temperatures) in different plots when $PVAD = 13^{\circ}$ were reached. ASW during
- the period between stage C and H (ASW_C-H), and between stage H and M (ASW_H-M) are also shown.

356

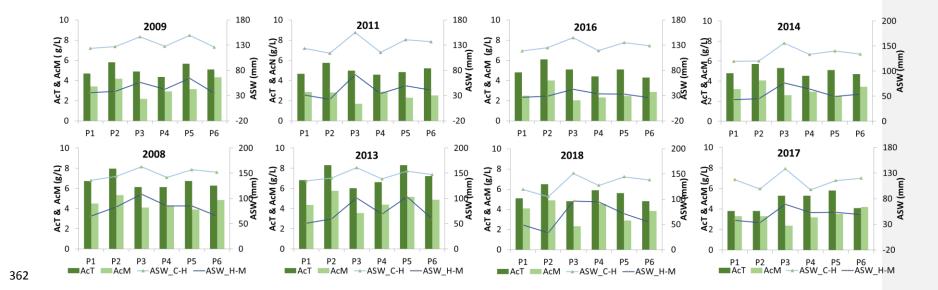


Fig. 5. Average values of total acidity (AcT) and malic acid concentration (AcM) recorded in the analysed years (2009, 2011 and 2016: dry years; 2014: intermediate characteristics; 2008, 2013 and 2018: wet years; 2017: very high temperatures) in different plots when $PVAD = 13^{\circ}$ were reached. ASW during the period between stage C and H (ASW_C-H), and between stage H and M (ASW_H-M) are also shown.

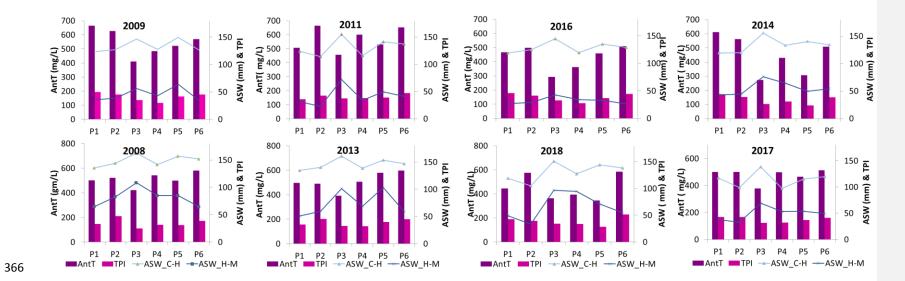
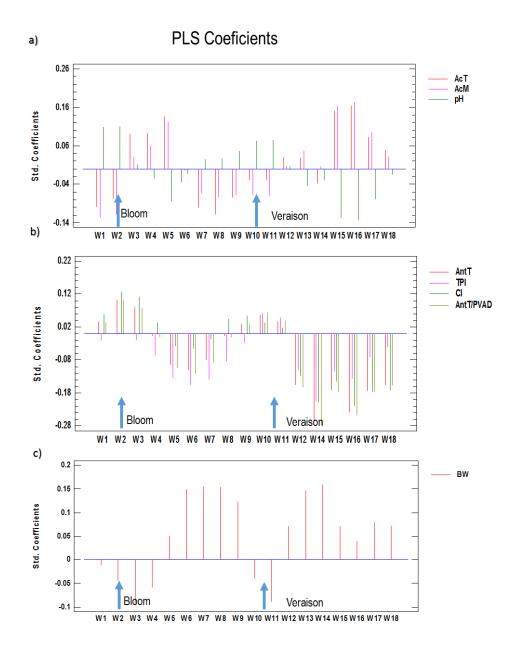


Fig. 6. Average values of total anthocyanins_(AntT) and total polyphenol index (TPI) recorded in the analysed years (2009, 2011 and 2016: dry years; 2014: intermediate characteristics; 2008, 2013 and 2018: wet years; 2017: very high temperatures) in different plots when $PVAD = 13^{\circ}$ were reached. ASW during the period between stage C and H (ASW_C-H), and between stage H and M are also shown.

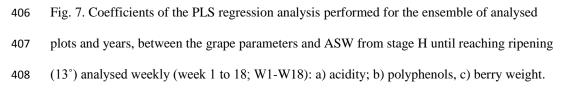
370 **3.5.** Relationship between water deficit and grape composition

371 The PLS regression analysis performed for the ensemble of analysed plots and years, between the grape parameters and ASW along the growing cycle allowed confirming the time for 372 373 which water availability plays an important role for grape quality. The fit coefficients for each 374 variable are shown in Fig. 7, in which the coefficients for each week starting after the stage H 375 and ending when the maturity was reached (weeks from 1 to 18 are presented). The results 376 shown in the figures corresponds to the number of components which gave the best fit and 377 lower error, being in all cases the variables significantly fitted at 95% (p<0.05). For the 378 variables related to acidity, two components were retained, and the percentage of the variance 379 explained was 65.2, 52.0 and 61.7%, respectively for AcT, AcM and pH. For the variables 380 associated with the phenolic compounds, one component was retained, and the percentage of the variance explained was 55.3, 65.1, 46.1 and 54.4%, respectively for AntT, TPI, CI and 381 382 AntT/PVAD ratio. Finally, BW was fitted using only one component and the percentage of 383 the variance explained was 27.5%. During the analysed years, bloom was reached 2 weeks 384 after stage H, veraison took place between 9 and 12 weeks after the stage H and maturity was reached between 17 and 20 weeks after stage H. 385

For the variables related with acidity, it was observed that ASW in the period between four 386 387 and seven weeks after the stage H and at the end of the ripening period increased acidity and 388 decreased pH, but there was a period in between in which the effect was negative or had not significant effect (Fig. 7a). The correlations were significant at 99% for the three parameters 389 390 (AcT, AcM and pH). Regarding the phenolic characteristics, it was observed that soil water 391 content in different periods within the growing cycle had influence on the final value of 392 anthocyanins as well as on the TPI and CI. The results were significant at 99% level for the 393 three parameters and it showed to be important before veraison and in particular during 394 ripening. It can be observed that the three parameters presented an inverse relationship with 395 ASW, which means that they increase when ASW decreases. As it was commented before, 396 ASW decreased very fast before veraison, reaching the conditions of moderate stress in 5 to 8 397 weeks after stage H, on average in the dry years. In wet years, the situations of moderate 398 stress were reached between 7 to 11 weeks after stage H. According to the results, the lowest 399 ASW in the weeks after veraison, when stress already existed, favoured anthocyanin concentrations and increased the TPI (Fig. 7b). The ratio anthocyanin/PVAD presented 400 401 similar relationship with ASW, thus is, it increased with water stress (Fig .7b). Finally, it was 402 observed that ASW had influence on berry weight in different time periods along the growing cycle, in particular in the period after bloom in which the berry is growing and then during 403 404 ripening (Fig. 7c).







410 **4. Discussion**

This research covered years with different rainfall amounts and distribution and also with differences in temperature, which allowed extracting information about soil water content and water availability under different weather conditions. During the period analysed (2008-2018), vines suffered different levels of stress during the growing season, but it was also confirmed the effect of high temperatures on grape composition.

416 It was observed that the soils usually maintained the maximum ASW up to stage H and from that time, ASW decreased very fast reaching values corresponding to 20% (considered as the 417 threshold to define moderate to weak water deficit of the maximum capacity) between 5 and 8 418 419 weeks after that stage H. In all plots, levels below 50% of the maximum holding capacity 420 were reached during the growing season, usually between bloom and veraison. For example, 421 for 2014, soil water reached the values corresponding to 50% of the maximum capacity in early July in P3, while it was reached in the middle of June in P1. In wet years, like 2013, in 422 423 which rainfall was about 20% greater than the average, the soils with the maximum water 424 holding capacity maintained soil water levels higher for longer time period. Besides, the ASW 425 below the 50% of the maximum, was reached at the end of July or at the beginning of August. 426 However, in the plots with lower holding capacity, this occurred in the third early part of July. 427 On the other hand, in dry years like 2009, 2011 or 2016, which received about 40% less of 428 precipitation than in the first case (2014), with about 30% of it recorded during the growing season, the differences between plots were smaller, and the 50% of ASC in the soil profile 429 was reached in all cases around the middle of June. At veraison, the ASW usually reached 430 431 very low values, around 10% of the AWC, except in the very wet years (e.g. 2013) (Fig. 4). 432 However, even in the driest years (e.g. 2009 or 2011), that minimum values were reached 433 before in the plots with lower maximum available water content. Nevertheless, for a given 434 year it happened up to 15 days earlier in some plots in relation to the rest, depending on soil435 characteristics.

The evolution of ASW and the minimum values observed were similar to that found by other authors (Pellegrino et al., 2005; Ramos and Martínez-Casasnovas, 2014). These authors expressed the ASW in relation to the AWC and they found values below 0.1 in some of the analysed years and locations, by the end of the growing cycle. Under these low values of ASW, the vines suffered from weak to moderate stress at veraison and during most time of the ripening period and it was confirmed that the reduced ASW at the end of the growing cycle influenced not only berry weight but also composition.

Lakso and Poll (2006) indicated that vines are sensible to water stress in different moments along the growing cycle. Water stress reduces shoot growth under any stress level, which could be beneficial in grapes for wine production, in particular when the vines have excessive foliar area. However, in the study case no water stress was shown during the first stages of the growing cycle. More important could be the effect on berry growth during the first weeks after bloom, which could affect the cellular division and can affect the rest of the cycle.

As it was commented before, in the study case the water deficit started in some plots just two 449 450 or three weeks after stage H. The situations of moderate stress, however, were recorded after 451 five weeks of stage H, even in the dryer years, and the highest water stress were observed at 452 veraison, in which soil water content was very low and ASW was below 10% in most years 453 and plots. According to Lakso and Poll (2006), water stress in the following weeks to 454 veraison is not as critical as it is at the end of the cycle, when it can produce both a decrease 455 in berry size and in sugar accumulation. However, Zulini et al. (2007), indicated that water 456 stress may affect the photosynthesis and lower yields may be produced because not all berries achieve the full ripeness. Berry weight was mainly related to ASW as was previously stated 457 (Ojeda et al., 2001; Reynard et al., 2011; Wenter et al., 2018; Ramos and Martínez de Toda, 458

459 2019), with higher values in the wettest than in the drier years, but it could be seen that there 460 were some specific periods in which the effect on berry growth was higher. Water status had 461 positive effects not only during the pre-harvest period but also in some periods before 462 veraison. In this study case, ASW had positive influence in almost all cycle. The coefficients 463 of the PLS were significantly high during berry growth, since two or three weeks after bloom, and then during the first weeks of ripening. The effect of this last period is consistent with that 464 465 reported by van Leeuwen et al. (2009), who indicated smaller berry size under water restrictions on the maturation. 466

467 There are only two periods in which the berry weight shows a negative relationship with the 468 ASW, two or three weeks after bloom and two weeks before veraison. The explanation could 469 be linked to the fact that, in these two periods, the balance between vegetative and 470 reproductive growth is more critical than in other periods, and the greater availability of water 471 would favour vegetative growth compared to the development of the berry. In the case of the 472 post-bloom period, it could also be influenced by the fact that there is a greater fruit set at higher ASW so, as the number of berries is greater, its unit weight would decrease. In the case 473 474 of veraison, a higher ASW would favour the vegetative growth, delaying the development of 475 the berries at the beginning of the ripening period.

476 In addition, several studies have pointed out the influence of water deficit on grape quality. 477 Water stress affects nitrogen metabolism and assimilation (Bertamini et al., 2004). In this 478 respect, Hochberg et al. (2015) indicated that water deficits increased amino acid content and 479 Valdés et al. (2019) pointed out the effect of pre-veraison vine water status on amino acid 480 concentrations. Other parameter that can be directly affected by water levels is the acidity. The highest acidity values were observed in the wettest analysed years. Cheng et al. (2014) 481 482 linked higher acidity to the wetter soils and higher acidity has been reported under excessive soil moisture (Jackson et al. 1993) and under well-watered grapes (Girona et al., 2009; 483

Reynolds et al., 2007), while Lopes et al. (2011) indicated that in treatments where soil water 484 485 content during spring was reduced, must titratable acidity experience a significant reduction. 486 Similarly, Peyrot des Gachons et al. (2005), de Souza et al. (2005) and dos Santos et al. 487 (2007) indicated lower total acidity due to water deficit and/or to changes in temperature and 488 sun-exposed berries in non-irrigated vines. Regarding the time along the cycle in which some effects were recorded on both total acidity and malic acid, the reason may vary. Higher water 489 490 available in the first stages of the fruit development, in which the berry is very small and 491 when acids are being produced, can have positive effect on the final acidity, which could 492 explain the observed positive PLS coefficients in that period. The negative PLS coefficients in the following period could be due to a dilution effect within the berry that increases in size 493 494 quickly. It was also found that higher ASW during ripening favours the increase in acidity. This result agrees with that found by Ramos and Martínez de Toda (2019), in a study who 495 496 covered almost the whole Rioja vine growing area, who found the highest acidity values in 497 the wettest years, in which an important amount of water was accumulated not only in the 498 period bloom to veraison but also in the period veraison to maturity. In that case, in addition, 499 the lower temperatures recorded in the wetter years could have also slow malic acid 500 combustion in particular during ripening.

501 The positive effect of water stress on anthocyanins and total phenols observed in this study 502 case are in agreement with observations from other authors (Hochberg et al., 2015; Cáceres-503 Mella et al., 2017; Ferrer et al., 2014; Ramos and Martínez de Toda, 2019), although the 504 different compounds may be affected in different ways depending the cultivars and rootstocks 505 (Berdeja et al., 2014; Hochberg et al., 2015). However, Niculcea et al. (2015) found that water 506 deficit decreased anthocyanins due to decreasing glucoside derivatives and increasing acetyl 507 and coumaroryl derivatives but increased flavonols in Tempranillo and decreased flavonols 508 and catechins in Graciano. Basile et al. (2011) indicated that anthocyanin and polyphenol **Con formato:** Español (alfab. internacional)

509 concentrations improved when no water stress occurred from bloom to fruit set, with mild water stress between fruit set and veraison, and with moderate to severe water stress in post-510 511 veraison. This is in agreement with the positive coefficients observed in the first weeks, and 512 with the negative coefficients observed after veraison. In this respect, Ferrer et al. (2014) 513 pointed out higher anthocyanin concentrations under mild to moderate water deficit during maturation. The only periods in which there was a discrete positive relationship between 514 water availability and phenolic compounds coincide exactly with the two periods in which the 515 ASW reduces the berry size, so we can say that the highest concentration of phenolic 516 517 compounds is due to the effect of ASW on berry size. In the 18 weeks studied, from stage H to maturity, there were only two short periods, of two or three weeks each (with the exception 518 519 of one of five weeks in the case of acidity), in which the effect of the water availability was contrary, although less intense, to the general effect during the rest of the vegetative period. 520 521 That is to say, the greater ASW in those two concrete periods decreased berry weight and 522 acidity and increased anthocyanins and other phenolic compounds. These two periods 523 correspond, in all cases, with bloom and with veraison, and the explanation could be related to the general physiological, and especially hormonal, changes that occurs in the vine in those 524 525 two stages: in both, the vegetative development it slows down in concurrence with 526 reproductive development; in one case to form the fruit and in the other case to start the 527 ripening period (Martínez de Toda, 1991).

Water deficit also affected positively the anthocyanin /sugar balance. Under high temperatures in summer, it can be observed a decoupling between anthocyanin and sugar content (Martínez de Toda and Balda, 2015). Nevertheless, the effect of temperature should be considered in addition to that of water availability. Sadras and Moran (2012), indicated that a moderate water deficit before veraison could partially restore the anthocyanin/sugar balance and Mori et al. (2005) indicated the effect of the night temperatures on anthocyanin

concentrations, with increasing values under cool nights. Similarly, Torres et al. (2017) 534 535 indicated that both factors (water deficit and high temperature contribute to modify metabolite 536 profiles of amino acids, anthocyanins and flavonols). In this study case, the PVAD was used 537 to define ripening, but the levels of anthocyanin varied from year to year, and it can also be 538 confirmed the effect of ASW on this ratio: the lowest the ASW the highest the anthocyanin/sugar ratio was, having influence on it the ASW in different periods along the 539 growing cycle. Regarding the effects of temperature on acidity, the lowest acidity found in the 540 warmest year, confirmed its effect, and the result agreed with those found in other studies 541 542 (Sadras et al., 2013; Martínez de Toda et al., 2019), in which it is indicated that water deficits 543 and high temperatures give rise to acidity lower than the average.

544

545 Conclusions

From this research we can conclude that the level of water stress and the time when it appears 546 under similar weather conditions varied between plots, due to differences in soils 547 548 characteristics and it has influence on grape composition. An increase in the available soil 549 water in most of the period between bloom and maturity increases berry size and acidity and 550 decreases the concentration of anthocyanins and other polyphenolic compounds. In particular, 551 an increase in available soil water between four and seven weeks after the stage H and at the end of the ripening period increased acidity and berry weight and decreased pH, while for 552 anthocyanins and other phenolic compounds an increase in available soil water after just 553 before veraison and in particular during ripening, decreases their concentrations. Under 554 climate change scenarios, in which temperature is predicted to increase, soil water available 555 could decrease due to higher evaporative demands, whatever the changes in precipitation can 556 be. In this respect, the zones located at higher elevation, which could have lower 557

558	temperatures, has been indicated as potential areas to mitigate the effects of climate change on
559	the vines. But additionally, the selection of the soil that can offer more favourable soil water
560	reserves and with higher availability should be considered.
561	
562	Acknowledgements
563	Authors thank the Consejo Regulador of Rioja DOCa by the information related to the plots
564	analysed in the research and the Government of La Rioja by the climatic information used in
565	this study.
566	Funding: The soil water measurements were done in the project RTA2009-00101-00-00
567	funded by INIA-MINECO, Spain, and the European Social Fund.
507	Tunded by INTA-INTIALEO, Spain, and the European Social Fund.
568	Declaration of Competing Interest: None
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	Defense
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