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Additional Information

# Effects of the irrigation regime on grapevine cv. Bobal in a Mediterranean climate

## I. Water relations, vine performance and grape composition.

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**Running head:** Irrigation regime Bobal

### Abstract

Climate change scenarios are predicting an increase in temperature as well as more scarce and torrential rainfall episodes. Due to this, an imbalance between grape technological and phenolic maturity is being observed detrimentally affecting grapes composition. In semi-arid areas, irrigation management is a main field practice to influence grape ripening. The goal of the present study was to investigate in *Vitis vinifera* L. cv. Bobal grapevine responses to three watering regimes: i) Rainfed, ii) deficit irrigation (DI) replacing only 35% of the estimated crop evapotranspiration (ET<sub>c</sub>) and ii) full irrigation (FI) replacing 100% ET<sub>c</sub>. In the mid-summer, rainfed grapevines showed different degrees of water stress determined by midday stem water potentials ( $\Psi_{\text{stem}}$ ) ranging from -1.1 to -1.4 MPa depending on the season. Rainfed plants had in all seasons less vigor and production and, at harvest, higher concentrations of total soluble solids (TSS) and grape phenolics compounds, as well as lower pH, with respect to the other water regimes studied. DI grapevines, generally, had intermediate values between Rainfed and FI, which presented extreme values of the studied parameters respect to Rainfed. The effects observed on grape color parameters and phenolic compounds with the Rainfed regime were mainly due to a dehydration of the berry, which lowered the yield and the weight of the berry compared to the irrigated

treatments. The lower TSS accumulation in the DI berries with respect to the Rainfed, will favour obtaining wines with lower alcohol content, currently more demanded by the consumers. Besides, despite the differences obtained between water regime treatments in the TSS accumulation, the extractability of the anthocyanins was similar, which is interesting since anthocyanin extraction from grapes is prerequisite to the formation of stable red wine pigments. Although the most convenient irrigation strategy might depend to the wine style to be obtained, DI is a strategy that can help to close the gap in the imbalance between the technological and phenolic maturity, positively affecting vine yield and performance with respect to the rainfed strategy.

### **Keywords**

Bobal, regulated deficit irrigation, water stress, yield, grape, polyphenols

### **1. Introduction**

With an increase in aridity and the frequency of extreme events predicted in the near future according to global climate models (IPCC, 2018), soil water availability may become a more important limiting factor in wine production and quality. Thus, the rise of temperatures and the decrease of precipitations due to climate change trigger advanced maturation, causing an imbalance between grape sugars and phenolic maturity (Poni et al., 2018), which affects grape composition and therefore, wine quality (van Leeuwen and Darriet, 2016). However, together with higher temperatures, higher concentrations of CO<sub>2</sub> are expected to increase biomass production (Moutinho-Pereira et al., 2009). However, vine productivity might be limited by rainfall distribution and water availability, especially at the end of the growth cycle (Fraga et al., 2016, 2018). Therefore, in order to adapt the viticulture to this changing situation, different management techniques, such as deficit irrigation (DI), can help to stabilize yield and maintain or improve wine quality, as well as to increase water use efficiency (WUE) (Chaves et al., 2007; dos Santos et al., 2007; Galvez et al., 2014; Lanari et al., 2014; Cole et al., 2015; Bonada et al., 2018).

Deficit irrigation consists of applying water rates to replace only part of the potential vine evapotranspiration either during the whole season or only during some phenological periods previously established (Intrigliolo and Castel, 2010). However, the phenological stage when RDI is applied, in addition to the water stress intensity and duration posed by the irrigation system, determines the vinegrape growth behaviour as well as the grape composition and quality attributes. Thus, McCarthy, (1997) reported

that water stress during the period from fruit set to veraison heavily reduces fruit size. Intrigliolo et al. (2012) and Niculcea et al. (2014) found that water deficits negatively impact berry weight, especially when applied before veraison. Salón et al. (2005) in Bobal cultivar and, Intrigliolo et al. (2012) in Tempranillo, showed that the delay of irrigation start during the period before veraison, until reaching a moderate stem stress level (stem at midday of -1.0 MPa), allows good control of vegetative growth and a moderate reduction in berry size. This resulted in berries with a higher concentration of anthocyanins, being the remaining grape composition parameters (sugars, total acidity, pH, malic and tartaric acids and total phenols) similar to those of fully irrigated vines. However, they reported that a post veraison deficit irrigation strategy (irrigation of 25-50% of grapevine total requirements) and avoiding severe water stress (stem water potential < -1.4 MPa) did not affect the final berry size but reduced the accumulation of sugars in it and delayed the ripening. Likewise, Girona et al. (2009) showed that only moderate water stress at post-veraison on Tempranillo vines (stem water potential < -1.0 MPa) can have positive effects on grape composition. Moreover, authors as Ojeda et al. (2002), and Pellegrino et al. (2005) and Romero et al. (2016) found a substantial improvement in berry quality under DI treatment for decreasing yield and berry-size. Roby et al. (2004), Van Leeuwen et al. (2004), Salón et al. (2005), Ferrandino et al. (2014), Degaris et al. (2015) and Conesa et al. (2016), observed that the synthesis and concentration of phenolic compounds, soluble solids and anthocyanins were promoted by water deficit. However, other authors such as Walker et al. (2005) and Zarrouk et al. (2016) obtained contrary results with anthocyanins reductions in the grape skins and loss of colour in the wines compared to those of rainfed vineyards. In particular, Zarrouk et al. (2016) demonstrate that seasons with increased water stress, lead to a larger impact of high temperatures on the berry ripening and composition. Petrie et al. (2004) also reported reduction in berry and bunch weight, yield and sugar concentration and increases in phenolic compounds concentration when water deficit was applied at Shiraz grapevines in the post-veraison period but anthocyanin concentration was unaffected. They observed that although in the following season irrigation was returned to standard practice, yield was reduced in accordance with the previous season stress. Therefore, Petrie et al. (2004) reported that post-veraison water deficit has the potential to impact both yield and fruit composition during both the current and the subsequent season. According to Koundouras et al. (2006) and Bindon et al. (2011), the positive effects of water deficit in grapevines are directly related to wine quality components

such as colour, flavour and wine aroma due to an increment of skin to pulp ratio in berries. Besides, there is a wider consensus that pH and titratable acidity values are not greatly affected by irrigation (Sivilotti et al., 2005).

Bobal (*Vitis vinifera* L.) cultivar is a native variety of the Utiel-Requena Designation of Origin (Southeast of Spain), which covers 78% of the total surface of the red varieties planted in this grape-growing region (Méndez, 2005; Intrigliolo and Castel, 2010). Its main characteristics include its productivity, a large number of clusters and large size, high acidity and polyphenols levels, its color intensity and brightness, and fresh aroma, which give rise to good red and rosé wines. It is a vigorous and semi-upright plant, with high fertility, has medium-high production, large clusters, and long and creeping shoots. Bobal is very resistant to drought and it is better suited to loose and airy soils. In La Mancha region (central Spain), Bobal has been cultivated in vineyards where the soil is poor and yields are low to obtain high quality grapes (Gómez García-Carpintero et al., 2011). There, Bobal is cultivated in areas with specific weather conditions (warm summers, cold winters and limited rainfall), which could influence its aroma composition (Dugelay et al., 1993). However, although drip irrigation has been steadily increasing for its crop, there is little information published about the effect of the irrigation management on the Bobal vine performance and grape and must composition. To our knowledge, only Salón et al. (2005) studied the effect of drip irrigation on Bobal agronomic performance and on red and rosé wines quality in vines that were trained to an open vase system and wide vine spacing. Therefore, the main objective of this study was to investigate how different irrigation strategies could affect the agronomic and oenological behaviour of a Bobal vineyard under vertical shoot positioning and a general more intensive vineyard plantation system.

## **2. Material and methods**

### *2.1 Site description, soil and meteorological data*

The trial was carried out from 2012 to 2014 in a commercial vineyard located near Requena, Valencia, Southeast Spain (Latitude: 39° 29' N; Longitude: 1° 13' W; elevation above sea level: 750 m). The experiment was conducted with *Vitis vinifera* L. cv. Bobal grafted onto 161-49C Couderc rootstock. The grapevines were trained to a vertical trellis system on a bilateral cordon oriented North-South and were planted in 2002 at a spacing of 2.5 x 1.4 m (2,857 vines/ha).

The soil of the vineyard was a Typic Calciorthid with a clay-loam to light clay texture, highly calcareous and of low fertility. Soil depth was > 2 m and available water capacity was about 200 mm/m. The climate in the area is continental Mediterranean and semiarid. The Heliothermal index is 2,291°C corresponding to a temperate warm viticultural climate, with cool nights and moderately dry according to the classification system for grape-growing regions proposed by Tonietto and Carbonneau (2004). At the experimental site, the annual average values for the last 12 years of the reference evapotranspiration ( $ET_o$ ) and the rainfall were 1,127 mm and 380 mm, respectively. About 65% of precipitation occurs in winter time, thus during the grapevine dormant period. During the vegetative period (from April to end-September) of the study years, the average temperature, the  $ET_o$  (calculated using the Penman-Monteith formula described by Allen et al. (1998)) and the rainfall data were recorded (Table1).

## *2.2 Experimental design and irrigation treatments*

The experiment was a randomized block design with three treatments in four replications. There were then a total of 12 experimental plots with four plots per each treatment. Each of the replications had 35 plants spread over five consecutive rows of seven grapes each one, although only the three inner rows were used for sampling, and the two outer rows were used as edges. Since plantation time, the experimental vineyard was deficit irrigation with around 60 mm/season (standard irrigation rates in the area). From 2012, the three irrigation treatments explored were as follows: i) R, rainfed, which only received rainfall water, ii) DI, deficit irrigation controlled, where water was applied to replace only 35% of the estimated crop evapotranspiration ( $ET_c$ ), iii) FI, full irrigation, where water was not limiting for the grapevines, applying 100% of the  $ET_c$ . The  $ET_c$  was estimated using the crop coefficient ( $K_c$ ) according to Williams and Ayars (2005) considerations, thus at full canopy growth, the estimated  $K_c$  value to refill the potential water needs was considered to be 0.6;  $ET_c = ET_o \times K_c$ . The drip lines had emitters of 4 L/h grapevine. All treatments were fertilized at a rate of 30-20-60-16 kg/ha of N,  $P_2O_5$ ,  $K_2O$ , and MgO, respectively. Fields practices were those commonly used in the area, including shoot trimming applied after fruit set.

## *2.2 Water status determination*

The plant water status was determined at midday (11:30 to 12:30 hr solar) by measuring stem water potential ( $\Psi_{stem}$ ) on two representative vines per experimental

plot and one fully expanded leaf on the outer rim of the canopy per vine, using a pressure chamber (PMS Instrument Company, model 600, Albany, OR, USA). The leaves were placed in totally hermetic aluminum foil bags for at least 1 h prior to the measurement time from mid-May-June to End-September-October on a bi-weekly basis according to climatic conditions and the phenological stage of the plant.

The water stress integral ( $S_{\psi}$ ) was calculated for each treatment using the stem water potential data, according to the equation defined by Myers (1988), in which the  $S_{\psi}$  was calculated (equation 1) as the sum of the difference of average of two consecutive plant water potential measurements  $\Psi_{\text{stem}} (\bar{\Psi}_{i,i+1})$  and the maximum (least negative) value registered during the study period (in our case was  $c = -0.45$  MPa), multiplying it by the number of days in the interval between one measurement and the next ( $n$ ).

$$S_{\psi} = \left| \sum_{i=0}^{i=t} (\bar{\Psi}_{i,i+1} - c) n \right| \quad (\text{Equation 1})$$

The water use efficiency (WUE) was calculated as a ratio between yield and the volume of water provided in each treatment through irrigation and rainfall. This is a core subject of interest to secure sustainability in viticulture (Medrano et al., 2014).

### *2.3 Leaf area estimation and vigor parameters*

After veraison, five representative shoots per replicate were collected and leaf area was measured in the laboratory using a leaf-area meter (LI-3100, Lambda Instrument Corp., Lincoln, NE). Primary and lateral leaf area was kept separated. The number of shoots was determined on all the experimental vines to calculate vine leaf area by multiplying the average shoot leaf area (obtained using the allometric relationships between shoot length and leaf area) per the total number of shoots per vine.

Shoot longitude, shoot weight and shoot mass per grapevine were determined at pruning. Ravaz index was calculated by the ratio between mass of clusters harvested and mass of cane prunings expressed in kg of grapes of cane prunings.

### *2.4 Berry ripening sampling and oenological determinations*

During the ripening period (from berry color change-veraison to harvest), randomly samples of 300 berries per experimental plot were taken at bi-weekly intervals to determine technological maturity and phenolic parameters. Date of the first sampling

for each year was: 08/28/2012; 09/10/2013; 09/8/2014 and date of the second sampling was: 09/4/2012; 09/23/2013 and 09/15/2014. Parameters related to technological maturity were berry fresh weight, berry total soluble solids (TSS) concentration (expressed as °Brix), pH, and total acidity (expressed in g/l tartaric acid). Grape phenolic maturity parameters at pH 1 and pH3.2 (color intensity, anthocyanins, polyphenols, total polyphenol index (TPI) and tannins) were determined during ripening only in 2013 and 2014 seasons, while in 2012 these parameters were determined only at harvest.

Total soluble solids (°Brix) was determined using a manual refractometer (ATC-1, Atago, Tokyo, Japan). The titratable acidity and pH were measured by manual titrating with NaOH 0.1M to an end point of pH = 7.0 using an electronic pHmeter METTLER TOLEDO S20 (Mettler-Toledo, Ohio, USA), following regulated methods (OIV, 2003).

For berry phenolics extraction, Saint-Cricq de Gaulejac et al. (1999) procedures were employed. Briefly, in order to extract phenolic compounds, four replicates of 50 g of homogenized sample were used for each sample. Two samples were used for phenolics determination after 4 hours of maceration with 50 mL of HCl 0.1 N buffered at pH 1.0, while extractable values were obtained after 4 hours of maceration with a solution buffered at pH 3.2.

The pH 1.0 extraction implies the liberation, diffusion and solubilization of the most percentage of the phenolics compounds, due to the degradation of the skin cells. The pH 3.2 extraction methodology is comparable to the one used during a classic red vinification (Saint-Cricq de Gaulejac et al. (1999), Intrigliolo et al. (2016)). Phenolic composition of the extracts was determined with UV and visible spectrophotometry, with a UV-Visible JASCO V-530 spectrophotometer (JASCO Analytical Instruments, Maryland, USA). Colour intensity (CI) and Polyphenolic Index (TPI) were estimated using the method described by Glories (1978). Anthocyanins and polyphenolic content were determined using the method to Puissant-León described by Blouin (1992). Tannins concentration was estimated according to Sarneckis et al. (2006).

The difference of the studied polyphenols concentrations in the two extracts at pH 1.0 and pH 3.2 is an indicator of the fragility of the skin cell membranes and also of the polyphenols extractability. The extractability percentage, described by Saint-Cricq de Gaulejac et al. (1999) as the capability of anthocyanins or polyphenols liberation, result in a decrease when anthocyanins and polyphenols extractability increase during



the ripening period. For that reason, the lower values for extractability percentage, the more capability of anthocyanin and polyphenol extraction. According to Intrigliolo et al. (2016), anthocyanin extractability (EA) represents the amount of anthocyanins that cannot be extracted in winemaking, and is calculated by the difference between the total anthocyanins extracted at pH1 and the extractable anthocyanins at pH 3.2.

Besides, considering that the total polyphenols determined in the extract correspond mainly to the sum of anthocyanins, skin tannins and seed tannins, and that there is a relationship, more or less fixed, in the accumulation of anthocyanins and tannins in the skin, the amount of tannins contributed by the seeds to the wine, can be determined by the difference. During maturation, the percentage of ripeness of the seeds decreases due to the grapes mature, so the amount of tannins in the seeds decreases and their contribution to the polyphenolic concentration is lower. Thus, the phenolic seed maturity (PM), which represents the contribution percentage of the seed tannins to the wine phenolic richness, was determined according to Saint-Cricq de Gaulejac et al. (1999) methodology.

All the analytical determinations were realized by duplicate, so the results were the average of two analyses ( $n = 2$ ).

### *2.5 Harvest sampling and yield components*

At the optimum moment of grape maturation according to the parameters set by the Utiel-Requena D.O., on 10<sup>th</sup>, 30<sup>th</sup> and 29<sup>th</sup> September of 2012, 2013 and 2014 seasons, respectively, 20 grapevines were harvested by hand for each repetition. Number of clusters per grapevine, bunch weight and yield (kg/grapevine) were recorded (KERN, CH 15k20, Spain).

Samples of 600 grapes were randomly selected for each repetition and were divided into two set of 300 berries, one for analysing flesh and seed evaluation and aromatic compounds (see details in García-Esparza et al., 2020) and the other one for determining technological and polyphenolic parameters in the destemmed and crushed must samples, following the same methodology as set out in section 2.4. Organic acids (malic and tartaric) were analyzed by high-performance liquid chromatography (HPLC) following the procedures described by Romero et al. (1993).

All the analytical determinations were realized by duplicate, so the results were the average of two analyses ( $n = 2$ ).

## 2.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) with irrigation treatment and year as factors. Mean comparisons were performed using Duncan multiple range test when the differences were statistically significant at 95 % probability level ( $p < 0.05$ ). Across seasons, data were analyzed with irrigation treatment, season and their interaction as factors. The probability levels used were  $p < 0.05$  (\*),  $p < 0.01$  (\*\*) and  $p < 0.001$  (\*\*\*). Simple linear regression analysis was performed to explore relationships between parameters, using the SigmaPlot package (version 14; Systat software, Inc., San José, CA; USA).

The statistical analysis were carried out using SPSS (SPSS Inc., Chicago, IL) for Windows, Version 11.5.

## 3. Results and discussion

### 3.1 Water balance components and vine water status

According to the data shown in Table 1 and in the Supplementary graph Supl. 1, on temperature, reference evaporative demand of the plants (ET<sub>o</sub>), rainfall and irrigation provided in each year, the three studied vintages were different. Thus, 2014 presented the most severe drought conditions, with lower rainfall and higher evaporative demand. Therefore, in this season, the irrigation started earlier (May 5) than in the other two vintages 2012 and 2013. From the 1<sup>st</sup> of April to the day in which the irrigation started, the rain recorded was 55.7, 112.3 and 4.6 mm in each season, for 2012, 2013 and 2014 respectively. However, in 2014, in relation to plant water status and according to the values indicate by Acevedo-Opazo et al. (2010), even the Rainfed plants showed moderate values of stress ( $\Psi_s < -1.2$  MPa, day 205) (Fig. 1). By contrast, in 2013 the irrigation began later (May 29) because the rainfall during pre-veraison was sufficient to increase the water available to the plants. Only at the end of the cycle, the plants under Rainfed treatment had some moderate stress (Fig.1) reaching similar  $\Psi_s$  values than in 2014 but only by the end of the season. On the other hand, 2012 vintage had an intermediate climate behaviour between the other two years. Although the rains fallen in spring allowed to reload with water the soil profile, in this year the irrigation started later (June 12), the scarce rains fallen during the summer resulted that the plants from the Rainfed treatment showed severe stress ( $\Psi_s = -1.29$  to  $-1.45$  MPa) during most part of their pre- and post-veraison and ripening period (from

2<sup>nd</sup> July to 3<sup>rd</sup> September) (Fig. 1). Besides, except for specific periods in 2014 (from 11<sup>th</sup> to 22<sup>th</sup> August) when the stem water potential of the DI plants was lowered ( $\Psi_s = -1.25$  MPa) and approached to those values presented by the Rainfed plants, during the rest of the growing cycles, the plants under the DI treatment did not suffer of water stress. The same occurred with the plants under the FI treatment that were the ones that presented the highest stem water potentials throughout the three studied years (Fig. 1).

Similar as observed by Intrigliolo et al. (2016) in their study with Cabernet cultivar, in 2012, the crop water use efficiency (WUE) decreased with increasing water application (DI and FI) (Table 2). However, in 2013 and 2014, the driest and rainnest seasons, respectively, WUE differences between irrigation treatments were not found probably because the positive mid-term effects of irrigation application on the 2014 vine crop level. Flexas et al. (2010) reported that WUE can be modified by the management techniques and timing of water availability. According to Simonneau et al. (2017), WUE is a parameter that depends on the genetics and the scions×rootstocks combinations used. Medrano et al. (2014) reported that the effects of deficit irrigation strategies in WUE are not conclusive and that many factors as genotypes, environment, soil and crop management can influence in plant behaviour to deficit irrigation strategies, mainly in relation to WUE. These authors also observed that there are cultivars reputed as more adapted to drought-prone conditions, which presumably should also present high WUE.

### *3.2 Yield and its components*

In all seasons, the most irrigated treatment (FI) had higher yield than Rainfed grapevines, being the yield of the Rainfed treatment in 2014 quite lower than the rest of the treatments (Table 2). This matches with the results obtained by other authors such as Williams (2010), Chalmers et al. (2010), and Intrigliolo et al. (2016). Thus, in general, the yield decreases as water availability does (Fig.2a,  $R^2 = 0.70; 0.57; 0.74$ , for 2012, 2013 and 2014 season, respectively), although the response varies depending on the irrigation dose, climatic conditions and the period of application. For instance, Yu et al. (2020) observed that early season water deficit irrigation (prior to veraison) had higher probability to decrease yield than later season water deficit irrigation (post-veraison to harvest). The number of clusters per grapevine and the cluster and berry weights also had the same trend as the yield; with higher values in grapevines under irrigation regimes (DI and FI). Although on the first season (2012) the number of clusters had not

significant differences between treatments (Table 2). This could be related to the fact that the higher budburst is a consequence of the improved water status during the previous season and, in 2011, all the grapevines had the same water status.

The increases in yield produced by irrigation were mostly due to the berry size, as also observed by Salón et al. (2004) in their Bobal and Tempranillo trials, where yield increased linearly with water application. In the present research, berry weight was the main yield component affected by water availability ( $R^2 = 0.83, 0.35$  and  $0.88$  in 2012, 2013 and 2014, respectively) (Fig. 2b) in agreement with Esteban et al. (1999), Salón et al. (2005), Baeza et al. (2007) and Intrigliolo et al. (2016). A significant effect of the year by treatment interaction was observed on cluster number per grapevine, cluster weight and berry weight, suggesting that the effect of the irrigation regime on these parameters was different among seasons (Table 2). Thus, these productive parameters were influenced by the vintage, being 2013, the coldest and the wettest year, where the cluster weight values in all treatments were lower compared to the other two study seasons. However, in 2013, the berry weight was similar between treatments with lower water availability and FI, probably due to the effect of the spring and summer rains in this year. Moreover, it is important to highlight that, although the yield between grapevines in 2012 and 2013 did not show significant differences between these years, the weight of the bunch was of the order of 3 (Rainfed and DI samples) and 4 (FI ones) times higher in 2012 compared to those of the 2013 year. Besides, the number of clusters per grapevine in the Rainfed treatment in 2012 was greater than in 2013 (Table 2). Girona et al. (2006) also shown significant reductions in the number of clusters after three years of severe deficit irrigation and in non irrigated vines and Shellie (2006) found a yield reduction associated with fewer cluster per vine under DI treatment.

On the other hand, the cluster fertility (number of berries per cluster), was not affected by water availability (Table 2), similarly to what reported by Baeza et al. (2007). These authors suggested that this could be because the flower differentiation is carried out near budburst when all treatments were well supplied with water from the winter rains. Etchebarne et al. (2010), in their Grenache noir vines, observed that the difference from one year to the next in berry number per cluster was most probably a consequence of variations in fruit set due to the weather conditions in each year, and not as a result of the irrigation treatments applied after berry set. However, authors like Keller et al. (2010) explained that high temperatures around budburst can lower the number of flowers per inflorescence, which could occur in the non irrigated treatment,

whose lesser vegetative development could modify the microclimate of the bunch increasing its temperature. Bergqvist et al. (2001) reported that high temperatures and high light intensity during the floral initiation process can increase the number of inflorescences, and Matthews and Anderson (1989) specified that a water deficit during this period can have strong opposite effects.

### 3.3 Vigor and vegetative growth parameters

The canopy components were affected by the irrigation treatments (Table 3); shoot weight and longitude ( $R^2 = 0.59$  and  $0.63$ , in 2013 and 2014, respectively) and pruning weight per grapevine ( $R^2 = 0.61$  and  $0.77$  in 2013 and 2014, respectively) were higher when irrigation treatments were applied (Figs. 2c and 2d, respectively). This suggests that these growth processes are very sensitive to water stress, although this also depends on the variety (Roby and Matthews, 2004 and Romero et al., 2013).

Similarly, leaf area per vine was affected by the irrigation treatment, increasing in the FI grapevines respect to those of the other treatments (Table 3). In 2014, the warmest and driest year of the study (Table 1), the differences in leaf area between Rainfed and FI vines, were statistically very considerable as can also be observed in the shoot total longitude (Table 3). Etchebarne et al. (2020) did not find irrigation effect on leaf area, mainly because the secondary shoots were removed and no compensatory growth seemed to occur for the primary shoots. Besides, the redistribution of grapevine reserves plays a role attenuating the effect of leaf area reduction (Candolfi-Vasconcelos et al., 1994). Candolfi-Vasconcelos and Koblet, (1990) reported that berry size increases when leaf area increases and, according to Dokoozlian and Hirschfeld (1995), when berry number decreases.

The Ravaz index (yield:vigor ratio) was not affected by the irrigation treatments (Table 3). This could be because the increase in yield due to irrigations was in most part compensated by the higher vine growth of the irrigated vines. Although this parameter differs with the grape variety, in order to obtain balanced grapevines, García-Escudero et al. (2006) suggested that for cv. Tempranillo in the climatic conditions of AOC Rioja region, the optimum values of Ravaz index ranged between 4-7, while Smart and Robinson (1991) recommend for *Vitis vinifera* L. values between 5 and 10. In our case, most of irrigated grapevines had Ravaz index around 7, without significant differences between watering regimes. Thus, the management and pruning of the vineyards were the same in all the treatments and should aim at the development of

balanced grapevines, ensuring stable yields over consecutive crops and without compromising the quality of the grapes. Therefore, in addition to the shaded atmosphere created in the area of the bunches and their conditions conducive to the development of diseases in berries (Dokoozlian and Kliewer, 1996), an excess of vigor in the vine can reduce the accumulation of sugars and phenolic compounds in berries, resulting from competition for assimilates between vegetative growth and bunch ripening. In this context, bunch ripening is delayed, which leads to problems in the ripeness of the berries, in the aromas and other compounds that can be sensitive to passed on to the wine (Pérez-Álvarez, 2015).

### 3.4 *Grape enological parameters.*

#### 3.4.1 Technological maturity

The date of harvest was twenty days before in 2012 (September, 10<sup>th</sup>) than in 2013 (September, 30<sup>th</sup>) and 2014 (September, 29<sup>th</sup>) seasons. However, the grape total soluble solids (TSS) accumulation (expressed as °Brix) varied between seasons (Tables 4, 5, and 6). Matthews and Anderson (1988) also observed the same behaviour and reported that water deficit might alter the onset or duration of the ripening period. Thus, at harvest, TSS was higher in 2012, even in FI grapes, than in the other seasons (Table 6). As aforementioned, year 2013 was the coolest year with the lowest ETo and the highest rainfall throughout growing cycle. Indeed, only the Rainfed and DI grapes in 2012 and the Rainfed in 2014 reached the target soluble solids concentration of 22 °Brix at the harvesting time (Table 6), being the TSS in the Rainfed treatment at harvest by about 1 °Brix higher than the values of DI and 1°Brix higher than those of the FI samples. Therefore, the reduction in berry weight and yield (Tables 2-3) caused by Rainfed or DI *versus* FI treatment resulted in an advanced maturation. Thus, two factors could explain the accumulation of TSS; a greater water deficit that advances the berry maturation, leading to a lower total acidity (TA) (Escalona et al., 2003) or a dehydration of the berry. In our study, the first case could be observed in DI grapes, especially in 2014, when TA was reduced compared to the other treatments. However, the increase in TSS in the case of the Rainfed grapes, could be due to a dehydration of the berry, which presented the lowest weights throughout the ripening process (Tables 2, 4, 5 and 6). Esteban et al (1999) reported that the TSS concentration was reduced when the dilution, caused by the berry growth, is faster than the increase in sugar transport into the berry,

in the same way that irrigation treatments were more conducive to dilution than in the Rainfed regime, because of larger berry growth (Table 2). Williams (2012) and Intrigliolo et al. (2016) also found that berry maturity is delayed and soluble solids concentration decreased with increasing water applications. Grapes with a high sugar content may lead to residual sugars in the fermentation responsible for impacting on the wine organoleptic characteristics. Therefore, in wineries, a current objective pursued is to reduce these soluble solids which is attempted through various cultivation practices such as DI or, already in the winery itself, through coupages or de-alcoholization processes (Intrigliolo et al., 2016).

In 2012 and 2014, the must pH showed correlation with water stress integral ( $R^2 = 0.58$  and  $0.31$ , respectively) (Fig. 3) confirming that must pH responses to the irrigation regime are dependent on several vine and edapho-climatic factors (Williams and Matthews, 1990). In all the seasons, at harvest time, the Rainfed treatment had the highest TSS (°Brix) and FI the lowest. On the contrary, the pH reached the lowest values in Rainfed treatment and the highest in FI (except in 2013 when there were no differences in pH between treatments) (Table 6). Etchebarne et al. (2010) observed that the pH was not affected by either leaf area:fruit ratio or soil water availability. In contrast, Esteban et al. (1999) found more differences in pH as distinct differences in soil water availability occurred during different growth seasons. According to Hrazdina et al. (1984), changes in the pH of the grape berries are caused by metabolism of the major acids and accumulation of cations, which transform the free acids into their corresponding salts.

The different environmental conditions and crop levels, probably affected the season effect of irrigation regime on must acidity (Tables 4-6). In 2012 and 2014, with drier conditions than in 2013, irrigation treatments decreased acidity, most likely because the Rainfed berries were more ripen (Supl. 2a) and had lower pH at harvest (Table 6, Supl. 2b). This did not match with those results observed by the majority of authors who investigated the response of total acidity to vine water supply, such as Estaban et al. (1999) which found higher acidity at harvest for the irrigated treatment (as occurs in our case in 2013) or Etchebarne et al. (2010) which grapes from Grenache noir grapevines with a favourable water status accumulated more organic acids at maturity.

At harvest, the tartaric acid content was higher in Rainfed samples in the three seasons. Thus, the higher acidity observed in 2013 in the irrigated samples could be mainly due to the highest malic acid concentration obtained in FI samples this year. In

general, malic acid is the organic acid which more contributing to changes the acidity (Romero et al., 1993) and which degradation is usually higher in non-irrigated vines because of less cluster shading by leaves and increased cluster exposure to direct solar radiation (Intrigliolo et al., 2010). Tartaric acid was more stable than malic acid (Esteban et al., 1999), and at harvest, its concentration were higher than malic acid (Table 6), as also observed by Esteban et al. (1999) in their study, while they reported that before veraison the maximum concentration of these organic acids, is reversed. The tartaric acid is difficult to metabolize, due to both its resistance to combustion at high temperatures and its propensity to form salts, which are not easily degraded by any known enzymes (Iland and Coombe, 1988). The application of irrigation altered the balance between malic and tartaric acid, increasing the former and decreasing the latter. Thus, the decrease of tartaric acid concentration with the irrigation treatments was determined by the dilution due to the increase in berry size respect to Rainfed berries. However, the malic acid content was higher under irrigated conditions (except in 2014), probably due to the lower solar radiation and temperature reached during ripening in cluster area respect to Rainfed grapevines. Rainfed grapevines shown lower shoot pruning weight and also had lower leaf area index (Table 3) than irrigated plants, with higher direct solar incidence that increases the temperature on Rainfed cluster zone, facilitating further malic acid degradation. This may aid in explaining the higher malic acid concentration under irrigated treatments compared to Rainfed in spite of the dilution effect in the irrigated treatment due to greater berry growth, as also observed Morlat et al. (1992), Esteban et al. (1999) and Intrigliolo et al. (2010).

#### 3.4.2 Phenological maturity

Throughout the ripening process, the interaction year by treatment was significant in most of the color, anthocyanins and tanins parameters measured, indicating that the season affected the must phenological parameters (Tables 4 and 5). However, at harvest, this interaction was not significant in the most of the must phenological parameters, suggesting that the effect of the irrigation regime on these parameters was not different between seasons (Table 6). In most of the color parameters measured at harvest at pH 3.2 (value close to that of the pH during fermentation), their highest amount was observed in Rainfed treatment (which were the berries that also had the greatest sugar accumulation at harvest), DI samples had intermediate values, and



samples of FI treatment showed the lowest color parameters content. Thus, it seems that a more severe water restriction favored a better grape maturation and increased must color intensity, polyphenols, anthocyanins, and tannins concentration. This could be consequence of both, an increase of expression of genes involved in synthesis of the phenolic pigmentes in berries by the water stress, as observed Castellarini et al. (2007) and Intrigliolo and Castel. (2010) , as seems to have occurred in 2013 when all the treatments had the same berry weight, maybe because the spring rainfall blunted the effect of the irrigation regimes or a dilution effect. Besides, it can also be seen that the color intensity, total polyphenols, anthocyanins and tannins concentration increase with the maturity of the grapes at harvest (Table 6).

However, although the polyphenols, TPI and tannins were influenced by the irrigation treatments, their evolution pattern through the ripening stage was different between the three seasons (Supl. 2 c, d, e and f). Thus, color parameters showed a gradual increase in 2012 and 2013 season but with a sudden increase in 2014 (after 7 days of accumulation) and to a subsequent decrease until the final content is achieved (at harvest) (Supl. 2 c and d). This also occurred with anthocyanin biosynthesis that was consistently increased throughout ripening (except in Rainfed and DI samples of 2014, the driest season) (Supl. 2e). By contrast, in the case of the tannins (Supl. 2f), in 2013 and 2014, their accumulation reached a maximum near the anthocyanins maximums but later its content remains almost constant throughout the grapes ripening until harvest. Thus, the accumulation of tannins was essentially complete by veraison, as also observed Bucchetti et al. (2011). Total tannin concentration was increased with Rainfed and DI compared to FI vines, as well as reported Romero et al. (2013).

However, despite finding higher concentrations of anthocyanins in grapes under treatments with water restriction, as observed Ginestar et al. (1998), Choné et al. (2001a,b), Ojeda et al. (2002) and Acevedo-Opazo et al. (2010), in the present experiment the percentage of the anthocyanins extractability (AE) only was higher in 2013 season in Rainfed respect to FI treatment. At harvest in this 2013 season, the grapes of Rainfed had higher TSS than the grapes from irrigated treatments (Table 6). Nevertheless, the percentage of maturation that had the seed at harvest was lower in the Rainfed treatment respect to those of the irrigated ones, which indicates that the seed tannins extractability was higher in the non-irrigated grapes than in the irrigated ones (Table 6). In 2013, the high seed ripening percentage of the FI grapes (Table 6), plus the anthocyanins increasing trend during the ripening (Supl. 2e), and added to the fact that

by the date of harvest of all the treatments, these grapes were the ones that reached the lowest accumulation of sugars, may reveal that the phenolic maturity was still incomplete. In 2012 and 2014 seasons, despite the differences in TSS accumulation obtained between water regime treatments, the extractability of the anthocyanins was similar, which is interesting since anthocyanin extraction from grapes is prerequisite to the formation of stable red wine pigments (Unterkofler et al., 2020). Thus, the greater skin anthocyanins observed in Rainfed and DI treatments respect to FI (García-Esparza et al., 2020) favours the extraction of total anthocyanins and easily extractable anthocyanins as reported Ginestar et al. (1998). Thus, according to Glories and Agustin (1993), it is important to note that phenolic maturity consists of two parts: the determination of anthocyanin and tannin potential and the extractibility of these compounds during winemaking. Thus, skins rich in anthocyanins and easily extractable tannins and seeds relatively poor in tannins characterize a ripe harvest. Hence, the amount of anthocyanins and tannins are a precise indicator to determine the harvest date according to phenolic maturity. Therefore, these wines whose technological maturity arrived earlier, leaving the phenolic maturity incomplete, will have a marked vegetable character, astringency and will be more diluted, lowering substantially its quality. This could be avoided by monitoring the evolution of phenols as ripening proceeds and the grapes are harvested at their optimum sugar/total acidity ratio. Both, technological and phenolic ripening processes are complementary and have to be synchronized, which is what determines the quality of the wine. In order to mitigate the desynchronization of both ripening processes, specially accentuated at the global level as a result of climate change, deficit irrigation management is an interesting tool that could help us to mitigate it and that should be further studied.

#### **4. Conclusions**

Despite that the vintage effect influenced on the growth and vegetative and productive development of the Bobal vines studied, irrigation management by means of a deficit irrigation treatment (DI), in the studied conditions, has emerged as an interesting tool to transmit the maximum oenological potential to the wine. Therefore, water regime did not affect the stability of the colour of the wine over time. However, the DI treatment produced a reduction in productive yield and vegetative development compared to the unlimited irrigation (FI), not as marked as the Rainfed treatment. So, presumably, DI regulated better the temperature and microclimatic conditions reached

in the bunches, which is fundamental for both, the quality and the ripening of the grapes and for their optimum health status. Besides, grapes from DI regime achieved higher total soluble solids accumulation and total acidity, lower pH, and greater potential of phenolic compounds than grapes with the maximum irrigation treatment (FI) and without differentiating of anthocyanin extractability degree between irrigation treatments. The wine's acidic taste is essential, since, together with the polyphenols, it counteracts the sweet taste of ethanol. Therefore, DI is a managing irrigation that can be interesting from an oenological point of view because contributes also to achieve a more favourable microbial and colour stability of the wines than that from berries coming from the other irrigation regime. In addition, with this controlled deficit irrigation system, the problem of water scarcity, which is faced by an increasing number of countries, especially those with a Mediterranean climate, is taken into consideration.

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## Tables

**Table 1.** Summary of the water balance variables in the Bobal vineyard for each experimental season (2012, 2013 and 2014). Temperature, reference evapotranspiration (ET<sub>o</sub>), annual rainfall, rainfall from 1<sup>st</sup> April to 30<sup>th</sup> September of each year and irrigation application in the treatments watered at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration (ET<sub>c</sub>) are shown.

Water balance variables	Period	Irrigation treatments	Year		
			2012	2013	2014
Average temperature (°C)	Annual		13.7	13.7	14.83
	April- 30 <sup>th</sup> Sept		19.6	18.8	20.2
ET <sub>o</sub> (mm)	Annual		1,220	1,212	1,281
	April- 30 <sup>th</sup> Sept		924	874	960
Rainfall (mm)	Annual		291	345	271
	April- 30 <sup>th</sup> Sept		114	208	119
Irrigation (mm)		Rainfed	0	0	0
		DI (0.35ET <sub>c</sub> )	90.8	74.1	125.2
		FI (1ET <sub>c</sub> )	251.4	224.9	375.1

**Table 2.** Water use efficiency (WUE) and productive parameters at harvest for Bobal grapevines in the rainfed application and in the treatments watered at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration (ETc) during each studied season (2012, 2013, 2014). For the data analysis across years, the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T × year factor was statistically significant at  $p < 0.05$  differences between treatment means were not explored.

Parameter	Treatment	2012	2013	2014	Average	Year	T x year
WUE (kg/m <sup>3</sup> )	Rainfed	4.45b	2.38a	2.10a	2.98a	**	**
	DI	2.81a	1.99a	3.16a	2.66a		
	FI	2.75a	2.51a	2.80a	2.69a		
Yield (kg/grapevine)	Rainfed	1.59a	1.54a	0.78a	1.29a	ns	ns
	DI	1.76a	1.65ab	2.42b	1.94a		
	FI	3.22b	3.15b	4.21c	3.53b		
N° clusters/grapevine	Rainfed	6.13a	5.19a	4.25a	5.19a	ns	*
	DI	5.94a	5.03a	6.34ab	5.77a		
	FI	6.84a	8.18b	8.28b	7.77b		
Cluster weight (g)	Rainfed	249.79a	88.56a	155.91a	164.75a	***	***
	DI	284.40a	95.71a	345.90b	242.00ab		
	FI	459.20b	114.50b	468.36c	347.35b		
Berry weight (g)	Rainfed	1.25a	2.90a	1.68a	1.94 a	***	***
	DI	1.54b	3.24a	3.11b	2.63 b		
	FI	2.45c	3.31a	3.72c	3.19 c		
Fertility (N° berries/cluster)	Rainfed	170.85a	87.44a	156.39a	138.40 a	***	ns
	DI	155.60a	104.06a	157.6a	139.09 a		
	FI	197.38a	94.30a	143.59a	145.09 a		

For each compound and year, different letters indicate significant differences between treatments at 95% ( $p < 0.05$ ) based on Duncan multiple range test. The probability levels used were  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*) and ns, not significant.

**Table 3.** Vigor parameters for Bobal grapevines in the rainfed application and in the treatments watered at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration



(ETc) during each studied season (2012, 2013, 2014). For the analysis of the data across years, the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T × year factor was statistically significant at  $p < 0.05$  differences between treatment means were not explored.

Parameter	Treatment	2012	2013	2014	Average	Year	T x year
Shoot weight (g/vine)	Rainfed	8.31a	6.14a	8.99a	7.82a	***	ns
	DI	11.39a	8.02a	15.08b	11.50b		
	FI	12.46 a	7.08a	13.97b	11.17b		
Weight of pruning wood (g/vine)	Rainfed	326.28a	345.09a	125.00a	265.45a	ns	ns
	DI	350.06a	436.02ab	329.00b	371.69b		
	FI	499.91a	495.53b	568.25c	521.23c		
Shoot total longitude (cm)	Rainfed	879a	877.15a	325.62a	685.59a	ns	ns
	DI	892.4a	1040.05ab	686.14ab	872.86a		
	FI	1114.60a	1145.05b	1659.05c	1306.23b		
Leaf area (m <sup>2</sup> /vine)	Rainfed	1.47a	1.12a	0.48a	1.07a	ns	**
	DI	1.49a	1.33a	1.00ab	1.27a		
	FI	1.86b	1.47b	2.94b	2.03b		
Ravaz index	Rainfed	4.85a	4.47a	6.45a	5.26a	*	ns
	DI	5.02a	3.76a	7.79a	5.53a		
	FI	6.96a	6.60a	7.38a	6.98a		

For each compound and year, different letters indicate significant differences between treatments at 95% ( $p < 0.05$ ) based on Duncan multiple range test. The probability levels used were  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*) and ns, not significant.

**Table 4.** Grape technological and phenological (pH 1 and pH 3.2) maturity parameters for Bobal grapevines in the rainfed application and in the treatments watered at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration (ETc) in the first maturation

sampling (August 29, 2012; September 10, 2013 and September 8, 2014). For the analysis of the data across years, the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T × year factor was statistically significant at  $p < 0.05$  differences between treatment means were not explored.

Parameter	Treatment	2012	2013	2014	Average	Year	T x year
<b>100 berries weight (g)</b>	<b>Rainfed</b>	112.20 a	272 a	153.0 a	179.62 a	***	***
	<b>DI</b>	140.06 b	302 b	316.5 b	252.18 b		
	<b>FI</b>	242.41 c	313 b	366.2 c	308.73 c		
<b>Total soluble solids (° Brix)</b>	<b>Rainfed</b>	21.788 b	18.3 c	23.21 c	21.17 c	***	***
	<b>DI</b>	21.125 b	17.6 b	20.81 b	19.85 b		
	<b>FI</b>	19.900 a	17.0 a	19.17 a	18.71 a		
<b>pH</b>	<b>Rainfed</b>	3.22 a	3.07 a	3.35 a	3.30 a	***	***
	<b>DI</b>	3.28 b	3.06 a	3.36 a	3.31 a		
	<b>FI</b>	3.38 c	3.05 a	3.41 b	3.33 a		
<b>Total acidity (g/l tartaric acid)</b>	<b>Rainfed</b>	8.11 c	7.6 a	5.86 b	7.21 a	***	***
	<b>DI</b>	7.23 b	8.4 b	5.67 a	6.97 a		
	<b>FI</b>	6.73 a	8.9 c	6.04 b	7.22 a		
<b>Colorant intensity pH1</b>	<b>Rainfed</b>		40 c	46.63 c	50.52 c	***	***
	<b>DI</b>		31 b	34.63 b	39.60 b		
	<b>FI</b>		21 a	23.72 a	23.28 a		
<b>Colorant intensity pH3.2</b>	<b>Rainfed</b>		13.3 c	12.22 c	14.50 c	*	**
	<b>DI</b>		10 b	7.40 b	10.47 b		
	<b>FI</b>		7.7 a	4.89 a	6.34 a		
<b>Anthocyanins (mg/l) pH 1</b>	<b>Rainfed</b>		969 c	998.9 c	1051.03 c	ns	***
	<b>DI</b>		796 b	597.6 b	781.43 b		
	<b>FI</b>		582 a	368.4 a	483.26 a		
<b>Anthocyanins (mg/l) pH 3.2</b>	<b>Rainfed</b>		388 c	352.8 c	425.20 c	ns	***
	<b>DI</b>		307 b	195.7 b	309.86 b		
	<b>FI</b>		243 a	137.8 a	201.99 a		
<b>Anthocyanins extractability (%)</b>	<b>Rainfed</b>		59 a	64.24 a	-	***	**
	<b>DI</b>		61 b	66.81 a	-		
	<b>FI</b>		63 b	63.78 a	-		
<b>Polyphenols (mg/L) pH 1</b>	<b>Rainfed</b>		3258 c	3312 c	3199.48 c	**	ns
	<b>DI</b>		2811 b	2533 b	2647.15 b		
	<b>FI</b>		2256 a	2083 a	1978.72 a		
<b>Polyphenols (mg/L) pH 3.2</b>	<b>Rainfed</b>		1942 c	1701 c	1863.71 c	***	***
	<b>DI</b>		1753 b	1277 b	1586.60 b		
	<b>FI</b>		1533 a	1052 a	1253.58 a		
<b>TPI pH1</b>	<b>Rainfed</b>		56 c	43.25 c	51.35 c	***	***
	<b>DI</b>		46 b	33.76 b	40.64 b		
	<b>FI</b>		34 a	29.90 a	31.13 a		
<b>TPI pH 3.2</b>	<b>Rainfed</b>		32 c	34.74 c	34.97 c	ns	***
	<b>DI</b>		30 b	29.55 b	27.36 b		
	<b>FI</b>		25 a	24.81 a	23.10 a		
<b>Tannins (mg/l) pH1</b>	<b>Rainfed</b>		2416 a	3183 c	1143.15 a	***	***
	<b>DI</b>		2307 a	2106 b	720.85 a		
	<b>FI</b>		2277 a	1861 a	658.82 a		
<b>Tannins (mg/l) pH3.2</b>	<b>Rainfed</b>		2466 a	2286 b	-	***	***
	<b>DI</b>		2434 a	1602 a	-		
	<b>FI</b>		2341 a	1307 a	-		
<b>% Seed ripening</b>	<b>Rainfed</b>		55 a	58.88 a	-	***	**
	<b>DI</b>		59 ab	73.01 b	-		
	<b>FI</b>		63 b	70.93ab	-		

For each compound and year, different letters indicate significant differences between treatments at 95% ( $p < 0.05$ ) based on Duncan multiple range test. The probability levels used were  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*) and ns, not significant.

**Table 5.** Grape technological and phenological (pH 1 and pH 3.2) maturity parameters for Bobal grapevines in the rainfed application and in the treatments watered at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration (ETc) in the second maturation sampling (September 4. 2012; September 23. 2013 and September 15. 2014). For the analysis of the data across years, the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T × year factor was statistically significant at  $p < 0.05$  differences between treatment means were not explored.

Parameter	Treatment	2012	2013	2014	Average	Year	T x year
<b>100 berries weight (g)</b>	<b>Rainfed</b>	121.35 a	287.1 a	160.8 a	189.81 a	***	***
	<b>DI</b>	152.27 b	315.1 b	319.7 b	263.63 b		
	<b>FI</b>	254.61 c	328.1 b	370.4 c	316.64 c		
<b>Total soluble solids (° Brix)</b>	<b>Rainfed</b>	22.6 c	20.2 c	23.59 c	22.18 c	***	**
	<b>DI</b>	21.75 b	19.2 b	21.22 b	20.9 b		
	<b>FI</b>	20.75 a	18.4 a	20.00 a	19.73 a		
<b>pH</b>	<b>Rainfed</b>	3.24 a	3.12 a	3.54 a	3.29 a	***	***
	<b>DI</b>	3.31 b	3.11 a	3.54 a	3.31 a		
	<b>FI</b>	3.44 c	3.08 a	3.56 a	3.36 a		
<b>Total acidity (g/l tartaric acid)</b>	<b>Rainfed</b>	6.8 c	6.2 a	5.67 b	6.16 a	***	***
	<b>DI</b>	6.31 b	6.8 b	5.29 a	6.24 a		
	<b>FI</b>	5.51 a	7.6 c	6.04 b	6.24 a		
<b>Colorant intensity pH1</b>	<b>Rainfed</b>	62.05 c	35 b	64.96 c	59.72 c	***	***
	<b>DI</b>	48.25 b	28 ab	42.67 b	45.28 b		
	<b>FI</b>	24.84 a	24 a	25.19 a	28.49 a		
<b>Colorant intensity pH3.2</b>	<b>Rainfed</b>	16.54 c	13.4 c	13.02 c	16.32 c	***	***
	<b>DI</b>	11.69 b	10.4 b	9.72 b	12.01 b		
	<b>FI</b>	6.15 a	7.9 a	6.54 a	8.30 a		
<b>Anthocyanins (mg/l) pH 1</b>	<b>Rainfed</b>	1083.3 c	1007 c	1348 c	1241.47 c	ns	***
	<b>DI</b>	844.28 b	791 b	852.9 b	906.94 b		
	<b>FI</b>	499.13 a	691 a	487.9 a	582.74 a		
<b>Anthocyanins (mg/l) pH 3.2</b>	<b>Rainfed</b>	511.03 c	409 c	485.6 c	486.25 c	ns	***
	<b>DI</b>	390.65 b	337 b	349.2 b	369.31 b		
	<b>FI</b>	224.36 a	277 a	182.7 a	239.36 a		
<b>Anthocyanins Extractability (%)</b>	<b>Rainfed</b>	59.64 a	57 a	62.48 a	986.64 a	***	ns
	<b>DI</b>	57.43 a	58 b	59.15 a	945.29 a		
	<b>FI</b>	54.52 a	60 b	62.64 a	919.40 a		
<b>Polyphenols (mg/L) pH 1</b>	<b>Rainfed</b>	2968 c	3229 c	4399 c	3653.85 c	***	***
	<b>DI</b>	2396 b	2663 b	3760 b	3023.21 b		
	<b>FI</b>	1596 a	2371 a	3007 a	2375.68 a		
<b>Polyphenols (mg/L) pH 3.2</b>	<b>Rainfed</b>	1871c	1986c	2813c	2361.40 c	***	***
	<b>DI</b>	1397b	1745b	2473b	2102.27 b		
	<b>FI</b>	1175 a	1490 a	2066 a	1580.19 a		
<b>TPI pH1</b>	<b>Rainfed</b>	53.27 c	58 c	63.73 c	62.89 c	ns	***
	<b>DI</b>	42.16 b	49 b	45.16 b	49.59 b		
	<b>FI</b>	29.29 a	40 a	37.66 a	38.17 a		
<b>TPI pH 3.2</b>	<b>Rainfed</b>	36.42 c	36 c	43.13 c	39.86 c	***	***
	<b>DI</b>	25.50 b	30 b	36.14 b	31.49 b		
	<b>FI</b>	18.87 a	25 a	31.70 a	27.13 a		
<b>Tannins (mg/l) pH1</b>	<b>Rainfed</b>	2090 c	3045 b	3478 c	2078.6 a	***	***
	<b>DI</b>	1838 b	2652 a	2692 b	1976.91 a		
	<b>FI</b>	1395 a	2641 a	2200 a	1482.59 a		
<b>Tannins (mg/l) pH3.2</b>	<b>Rainfed</b>	1534 c	2506 b	2628 b	1720.60 <sup>a</sup>	***	***
	<b>DI</b>	1116 b	2149 a	2150 a	1467.73 a		
	<b>FI</b>	998 a	2143 a	1842 a	1275.18 a		
<b>% Seed ripening</b>	<b>Rainfed</b>	-	54 a	54.68 a	912.37 a	***	***
	<b>DI</b>	-	58 ab	61.47 b	761.59 a		
	<b>FI</b>	-	60 b	70.37ab	755.025 a		

For each compound and year, different letters indicate significant differences between treatments at 95% ( $p < 0.05$ ) based on Duncan multiple range test. The probability levels used were  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*) and ns, not significant.

**Table 6.** Grape technological and phenological (pH 1 and pH 3.2) maturity parameters for Bobal grapevines in the rainfed application and in the treatments watered at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration (ETc) at harvest sampling (September 10th. 2012; September, 30th. 2013 and September, 29th. 2014). For the analysis of the data across years. the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T × year factor was statistically significant at  $p < 0.05$  differences between treatment means were not explored.

Parameter	Treatment	2012	2013	2014	Average	Year	T x year
<b>100 berries weight (g)</b>	<b>Rainfed</b>	123.22 a	290.66a	168.40a	194.09 a	***	***
	<b>DI</b>	153.71 b	324.76b	311.76b	263.41 b		
	<b>FI</b>	254.87 c	331.66c	372.74 c	319.76 c		
<b>Total solid solubles (° Brix)</b>	<b>Rainfed</b>	23.47 c	21.4 c	22.16 c	22.51 c	***	ns
	<b>DI</b>	22.11 b	20.1 b	20.51 b	21.05 b		
	<b>FI</b>	21.31 a	19.3 a	19.47 a	20.05 a		
<b>pH</b>	<b>Rainfed</b>	3.26 a	3.39 a	3.49 a	3.38 a	***	***
	<b>DI</b>	3.35 b	3.37 a	3.51 ab	3.40 ab		
	<b>FI</b>	3.45 b	3.31 a	3.53 b	3.46 b		
<b>Total acidity (g/l tartaric acid)</b>	<b>Rainfed</b>	6.18 b	5.8 a	5.06 b	5.59 a	***	***
	<b>DI</b>	5.34 a	6.3 b	4.53 a	5.43 a		
	<b>FI</b>	5.4 a	6.9 c	4.83 ab	5.72 a		
<b>Tartaric acid (g/L)</b>	<b>Rainfed</b>	5.22 c	3.9 b	3.92 c	4.34 b	***	***
	<b>DI</b>	4.44 b	3.5 a	3.67 a	3.85 a		
	<b>FI</b>	4.20 a	3.6 a	3.71 b	3.86 a		
<b>Malic acid (g/L)</b>	<b>Rainfed</b>	1.02 a	2.3 a	2.12 a	1.79 a	***	**
	<b>DI</b>	1.17 b	2.9 b	2.62 b	2.13 b		
	<b>FI</b>	1.97 c	3.3 c	3.12 c	2.80 c		
<b>Colorant intensity pH1</b>	<b>Rainfed</b>	75.41 c	39 c	61.79 c	50.63 c	**	***
	<b>DI</b>	59.79 b	31 b	36.68 b	35.84 b		
	<b>FI</b>	35.81 a	24 a	21.74 a	23.23 a		
<b>Colorant intensity pH3.2</b>	<b>Rainfed</b>	20.61 c	14 c	11.6 c	13.47 c	***	ns
	<b>DI</b>	16.59 b	11.7 b	7.51 b	9.65 b		
	<b>FI</b>	11.12 a	9.5 a	6.21 a	7.55 a		
<b>Anthocyanins (mg/l) pH 1</b>	<b>Rainfed</b>	1301.4 c	1064 c	1042.0 c	1080.24 c	***	***
	<b>DI</b>	961.37 b	874 b	604.7 b	761.20 b		
	<b>FI</b>	575.00 a	749 a	393.9 a	571.91 a		
<b>Anthocyanins (mg/l) pH 3.2</b>	<b>Rainfed</b>	537.88 c	661 c	359.0 c	512.82 c	***	ns
	<b>DI</b>	409.36 b	887 b	226.5 b	389.89 b		
	<b>FI</b>	258.34 a	457 a	166.9 a	312.41 a		
<b>Anthocyanins extractability (%)</b>	<b>Rainfed</b>	58.42 b	35 a	44.31 a	46.72 a	***	ns
	<b>DI</b>	57.08 b	42 b	47.65 a	46.28 a		
	<b>FI</b>	53.89 a	44 b	47.63 a	44.99 a		
<b>Polyphenols (mg/L) pH 1</b>	<b>Rainfed</b>	3225 c	3314 c	3827 c	3591.72 c	ns	***
	<b>DI</b>	2485 b	2987 b	2976 b	3032.46 b		
	<b>FI</b>	1749 a	2864 a	2547.4 a	2706.10 a		
<b>Polyphenols (mg/L) pH 3.2</b>	<b>Rainfed</b>	2363 c	2692 c	1823 c	2183.59 b	***	ns
	<b>DI</b>	1735 b	2270 b	1383 b	1807.74 a		
	<b>FI</b>	1184 a	2044 a	1261 a	1652.57 a		
<b>TPI pH1</b>	<b>Rainfed</b>	64.25 c	72 c	57.61 c	66.75 b	***	ns
	<b>DI</b>	50.69 b	56 b	39.05 b	48.94 a		
	<b>FI</b>	36.68 a	53 a	30.59 a	41.86 a		
<b>TPI pH 3.2</b>	<b>Rainfed</b>	38.16 c	54 c	41.97 c	48.94 c	***	ns
	<b>DI</b>	27.06 b	45 b	32.3 b	39.37 b		
	<b>FI</b>	20.91 a	34 a	27.77 a	31.07 a		
<b>Tannins</b>	<b>Rainfed</b>	2894 b	3510 c	3103 c	2766.07 c	ns	***

<b>(mg/l) pH1</b>	<b>DI</b>	2656 c	3247 b	2589 b	2515.5 b		
	<b>FI</b>	2187 a	2695 a	2051 a	2163.99 a		
<b>Tannins (mg/l) pH3.2</b>	<b>Rainfed</b>	2497 c	2632 c	2415 b	2296.52 a	***	ns
	<b>DI</b>	2076 b	2456 b	1901 a	2281.18 a		
<b>% Seed ripening</b>	<b>FI</b>	1922 a	2277 a	1820 a	2048.50 a		
	<b>Rainfed</b>	59.64 a	47 a	54.55 a	48.09 a	***	ns
	<b>DI</b>	57.43 a	50 ab	66.77 b	56.19 b		
	<b>FI</b>	54.52 a	53 b	61.27 ab	57.52 b		

For each compound and year, different letters indicate significant differences between treatments at 95% ( $p < 0.05$ ) based on Duncan multiple range test. The probability levels used were  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*) and ns, not significant.