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Effects of the irrigation regime on grapevine cv. Bobal in a Mediterranean climate II. Wine, skins, seeds, and grape aromatic composition.

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Abstract

This manuscript complemts the data and information reported on Pérez-Álvarez et al. (2020) and focused on determining the effects on wine and grape skin, seed and aromatic composition of grapevine cv. Bobal in response to the irrigation regime (i) Rainfed, ii) deficit irrigation, DI and full irrigation, FI). The results showed that the deficit irrigation treatment can modulate some important parameters of the grape and wine colour and the aromatic composition of the grapes with respect to rainfed and/or unlimited irrigation. In general, alcohol concentration and total acidity of wines decreased with water application while berries weight increased. Wine colour, total phenolics and, anthocyanins increased when water application was restricted because of the effect of water stress on anthocyanins, tannins and colour parameters of the grape skins and seeds. The water regime did not affect the seed polymeric concentration values while the polymerization of grape skin tannins (higher mDP, aMW and %G) from the irrigated treatments, positively affected must astringency. Some aromatic precursors such as benzaldehyde, guaiacol, 4-ethylphenol, 4-vinylphenol, α-ionone, γ-decalactone, syringaldehyde, and vainillin increased in the irrigated treatments respect to rainfed. Benzanoic acid, 3-hidroxybenzaldehyde and octanoic acid content also increased respect to full irrigation treatment. These increases can favour metabolic pathways that enhance certain volatile aromas in the wines, affecting their sensory quality. Overall the results presented demonstrated the important role that irrigation regime has in modulating Bobal grapes and wine composition.

Keywords

Bobal, regulated deficit irrigation, tannins, anthocyanins, skin and seed polyphenols, aroma compounds

1. Introduction

In arid and semi-arid regions, irrigation is one of the main determining factors for grape quality and, as a consequence, for final wine composition. In this sense, severe water deficit might impair the vine photosynthetic activity, affecting the grapevine vegetative development and the overall performance (Koundouras, 1999). Therefore, the expected water scarcity in many winegrowing areas as a result of the climate change scenario might result in negative effects on wine quality such as inhibition of anthocyanin accumulation, loss of grape color and acidity, increasing pH, alcoholic degree, volatilization of aromatic compounds (producing grapes with a low aromatic content), and an increased risk of organoleptic degradation (Resco et al., 2016; Pons et al., 2017). Thus, in many winegrowing regions, it will be necessary to apply irrigation to maintain the sustainability of vineyards and to avoid severe vine water stress (Resco et al., 2016).

Soil, climate and agronomic management implemented on the vineyard, are closely linked to the fruit morphological development affecting berry size, and therefore the surface/volume ratio. This implies a modification in the amount of skins and seeds in relation to the size of the berry. Therefore, this modification implies a greater or lesser concentration of aromas and anthocyanins (located in the skins) tannins (mainly found in the seeds and also in skins and stem/rachis tissues), and acids and sugars (presented in the pulp cells). From a winemaking perspective, reductions in berry size are considered desirable, because the surface to volume ratio of small berries is higher than that of larger berries (Mirás-Avalos et al., 2019). However, the question remains whether the desirable effects of deficit irrigation (DI) on grape and wine phenolics occur because of enhanced biosynthesis (i.e., on a per berry basis), or due to enhanced concentration (i.e., on a fresh weight basis) (Cassasa et al., 2015). Thus, factors such as environmental conditions (Koundouras et al., 2006), grape variety (Kallithraka et al., 2006), and viticultural practices (Kyraleou et al., 2015) influenced the accumulation of plant secondary metabolites, including phenolic compounds in grapes. These compounds have been recognized as playing multiple roles in plant response to a wide range of biotic and abiotic stresses, in particular to water stress (Caldwell et al., 2003; Pinasseau et al., 2017).

From both, chemical and sensory point of view, the most important phenolic classes contributing to the quality of red grapes and wines are anthocyanins (glycosylated pigments mainly responsible for the color of red wine) and proanthocyanidins or condensed tannins (mainly responsible for the astringent and bitter properties of the wines) (Cassasa et al., 2015). Furthermore, tannins also modulate wine color via their covalent reaction with anthocyanins to form polymeric pigments, which are orange or brick-red pigments with astringent properties

(Somers, 1971). The intensity of astringency in wine is reported to be related to both berry tannin concentration (Kennedy et al., 2006; Mercurio and Smith, 2008) and composition (Vidal et al., 2003, Woollmann and Hofmann, 2013). Thus, some studies (Chira et al., 2011; Quijada-Morin et al., 2012) reported that the tannin composition exerted a stronger influence on wine astringency than the total amount of phenolic compounds, while others suggested that astringency is more correlated with grape total phenolic and tannin content than with tannin structural composition (Kyraleou et al., 2016). Besides, according to these authors, astringency was also shown to be dependent on the presence of galloyl groups (%G) and prodelphinidins (proanthocyanidins containing gallocatechin or epigallocatechin subunits), although data from different studies such as Chira et al. (2011), Woollmann and Hofmann (2013), Curko et al. (2014) and Kyraleou et al. (2016) are contradictory.

The influence of irrigation on the accumulation of anthocyanins in grapes has been more extensively studied than the irrigation effect on the accumulation of grape proanthocyanidins. In general, as a consequence of the impact of mild water deficit, several authors reported an increase of anthocyanin content, attributed to changes in berry skin-to-pulp ratio (Santestaban et al., 2011) or modifications in grape microclimate (Romero et al., 2010), and a qualitative modification of the anthocyanin pool when more detailed analysis were performed (Castellarin et al., 2007a,b; Bucchetti et al., 2011; Ollé et al., 2011; Hochberg et al., 2015). On the other hand, the effect of irrigation management on berry tannins accumulation has not been extensively reported. Besides, comparing cv. Chardonnay (Deluc et al., 2009) or cv. Syrah (Hochberg et al., 2015) to cv. Cabernet Sauvignon, the cultivar specificity of these responses has been reported. This may be related to hydraulic behavior or to differences in phenological stages (Hochberg et al., 2015) as early and late water deficit affect phenolic composition in different ways (Ojeda et al., 2002; Ollé et al., 2011; Casassa et al., 2015).

Thus, in general, when grapevines are subjected to water deficit, the studies reported an increase in antochyanins content and in total polyphenol index (TPI). For instance, in the Kyraleous et al. (2016) study, the Syrah berry skin anthocyanins increased when water limitation was applied, although they observed that differences were maximum 2–3 weeks after veraison and decreased thereafter to reach similar levels at harvest. Intrigliolo and Castel (2009) observed, in Tempranillo irrigated grapevines, that the effect of irrigation on grape color and anthocyanins are dependent on the timing and severity of water stress. In this sense, Castellarin et al. (2007a,b) found that water stress might positively affect the anthocyanins synthesis pathway. Matthews et al. (1990) and Nadal and Arola (1995), observed the effect of a post-veraison water stress in cv. Cabernet Sauvignon finding inferior levels of phenolic compounds, anthocyanins and tannins on less-stressed vines. Similarly, Salón et al. (2005) reported that supplemental irrigation in Bobal grapevines, decreased grape and wine phenolics. By contrast, in their Syrah studies, Ojeda et al.

(2002) observed that a severe water deficit before veraison provoked a decrease of anthocyanin synthesis and Romero et al. (2010) in Monastrell found that an extremely severe water stress was detrimental to the total grape phenolic concentration. For its part, Cassasa et al. (2015) observed over tree consecutive growing season on Cabernet Sauvignon grapes and wines, that the DI regimes mostly affected the concentration of skin and seed phenolics, suggesting that the impact of these techniques is rather indirect and based on a reduction of berry size.

Concerning berry tannins, reports on the effects of water availability are fewer and inconsistent. Tannins are present in skins, seeds and stems, although their composition varies somewhat depending on which part of the cluster they come from (Pascual et al., 2016). Seed tannins are made up of oligomers and polymers of three flavan-3-ol subunits: (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-gallate (Prieur et al., 1994), whereas skin tannins also have (-)-epigallocatechin and a minor concentration of (-)-epicatechin-3-gallate (Souquet et al., 1996). Therefore, seed tannins consist of only procyanidins, whereas skin tannins include procyanidins and prodelphinidins (Pascual et al., 2016). According to previous studies, water deficit is reported to have little direct effect on the accumulation of tannins in berries (Kennedy et al., 2000; Bonada et al., 2015). However, in Cabernet Sauvignon studies, Kennedy et al. (2000) reported water limitation decreased the amount of seed flavan-3-ols at harvest while, Chacón et al. (2009) observed that the concentration of flavan-3-ols and tannins in Merlot seeds increased with the magnitude of water deficiency. Genebra et al. (2014) found no impact of irrigation on the levels of Tempranillo seed tannins, although several genes of the biosynthetic pathway of flavan-3-ols were up-regulated, while Zarrouk et al. (2012) found an increasing trend of skin tannins with irrigation. Roby et al. (2004) and Koundouras et al. (2009) reported that water deficit did not alter the concentration of seed tannins in spite of its impact on berry weight in Shiraz and Cabernet Sauvignon studies, respectively. In this sense, Pastor del Río and Kennedy, (2006) reported that seed tannin concentration is also determined by seed weight and the number of seeds per berry. After examined the effect of four DI regimes on Cabernet Sauvignon grapes, Cassasa et al. (2015) reported that, there was no apparent effect of any of the deficit irrigation regimes on seed and skin tannin content, suggesting that tannin biosynthesis is not altered by DI. Besides, they observed that both the DI regimes and the growing seasons affected the proportion of tannin extracted from seeds, whereas none of these two factors affected the proportion of tannins extracted from skins. Indeed, the effect of water stress on grape phenolics is far from being consistent among experimental studies. Usually, it is not clear if the reported effects were caused by berry dehydration, a higher skin to pulp ratio, or a change in the compound metabolism (García-Esparza et al., 2018). Besides, according to Cassasa et al. (2015), the content of phenolics was season-dependent, implying that different growing seasons are associated with specific biosynthetic effects that alter the phenolic content and, potentially, extraction and retention into wine.

On the other hand, the wine aroma is complex and is one of its main organoleptic characteristics, being the final result of a long biological, biochemical and technological sequence. Wine aroma profile is mainly consequence of two important groups of compounds; the free fraction of the aromas that are the volatile compounds, and the bound fraction of aromas, which are the aromatic precursors. Therefore, the volatile compounds are terpenes, C13-norisoprenoids, benzenoids, C6 compounds and pirazines, with about 800 volatile substances coming from the grape and, the aromatic precursors, which are non-volatile and odorants, capable of releasing aromas under the influence of various factors (Bayonove et al., 2000). The aromatic precursors can be classified into two groups depending on if they are specific aroma compounds or not. Fatty acids, carotenoids and amino acids are considered non-specific and their profile is characteristic of the variety. Specific aroma precursors are defined as those compounds that can produce odorous volatiles by means of one or two fragmentations of the molecule, being the structure of the precursor still recognizable (Salinas, 2013). Glycosides, volatile compounds bound to cysteine and glutathionic compounds are considered specific precursors of aroma. However, only the glycosidic precursors are found in all the viniferas, constituting a potential reserve of aromas, which can be released both during fermentation and throughout the aging of the wines. In addition, unlike cysteine precursors and glutathionic, glicosidic precursors are stable and are released both by enzymatic action and by acid hydrolysis (Salinas, 2013). This complexity of the wine aroma makes it complicated to predict the aroma properties of a wine from a given compound alone, because its perception can be affected by other wine volatile compounds. Furthermore, the accumulation of aroma compounds in grapes is highly influenced by a large range of biotic and abiotic factors, among them, environmental factors, such as sunlight (Zhang et al., 2017), water availability (Bouzas-Cid et al. (2018a,b) and Vilanova et al. (2019a,b)) and viticultural practices as cluster thinning (Feng, Skinkis and Qian, 2017) or the application of plant growth regulators, such as abscisic acid (ABA) (Jia et al., 2018), jasmonic acid (D´Onofrio et al., 2018), among others.

Therefore, little information is published about the effect of the irrigation managements on the aroma composition of grapes and wines made from Bobal grapes. To our knowledge, only Salon et al. (2005) studied the effect of drip irrigation on Bobal agronomic performance and on red and rosé wines quality. Besides, according to these authors, the market acceptance of Bobal wines is based primarily on its high color intensity and tannin concentration. In addition, Sivilotti et al. (2020) reported about the importance of further studying the effects of the water regime on the skin and seeds tannins. In this manuscript we report the effect of different irrigation strategies over a Bobal vineyard, througout three consecutive vintages in a semi-arid climate, in order to have more knowledge about the skin and seed phenolic composition and, the aroma compounds of this red variety.

2. Material and methods

2.1 Site description and experimental design

The experiment was carried out in a commercial grapevine of *Vitis vinifera* L. cv. Bobal grafted onto 161-49C Couderc rootstock, located near Requena, Valencia, Southeast of Spain (Latitude: 39º 29´N; Longitude: 1º 13W; elevation above sea level: 750 m). Soil, climate data of the site during the three years of the study (2012-2014) and all details about the experimental field work are described in the companion paper by Pérez-Álvarez et al. (2020).

The experiment was carried out in a randomized block design with three treatments in four replications. Since plantation time, the experimental vineyard was deficit irrigation with around 60 mm/season. From 2012, the three irrigation treatments proposed in the plot were the following: 1) Rainfed, receiving only rainfall water, 2) DI, deficit irrigation controlled, where irrigation replaced only 35% of the estimated crop evapotranspiration (ETc), 3) FI, full irrigation, where water was not limiting for the grapevines, applying 100% of the ETc. The drip lines had emitters of 4 L/h grapevine.

2.2 Grapes sampling, winemaking process and oenological parameter analysis

For each repetition, 20 grapevines were harvested at the optimum moment of grape maturation, according to the parameters set by the Utiel-Requena D.O. and which are typical of the cultural practices in the area. Samples of 600 berries were randomly taken for each repetition and the weight of 100 grapes of each repetition was determined. Then, grapes were divided into two set of 300 berries, one for determining technological and polyphenolic parameters (see details in Pérez-Álvarez et al., 2020) and another one for analysing flesh and seed evaluation and aromatic compounds. Grapes were stored in isothermal containers to be taken to the laboratory, where they were kept at -20ºC until analytical determinations.

Following the harvest, grapes were destemmed and crushed to obtain the must. The winemaking process was performed according to the Utiel-Requena D.O. usual methodology. Briefly, microvinifications were fermented at about 25ºC in stainless steel containers, one for each repetition. All were inoculated with a commercial yeast strain and were maintained skin contact during 7 days, automatically punched every 4 h. Wine probable alcohol degree (% v/v), pH, total acidity (g/L tartaric acid), and malic acid (g/L) were analysed according to the methodology established by the OIV (2003). Lactic and citric acids (g/L) were also analysed enzimately (Miura One, Tecnología Difusión Ibérica, Barcelona, Spain). Colour intensity (OIV methologoy), and total polyphenol index were analysed according to Ribéreau-Gayon et al. (2000). Wine

anthocyanins (mg/L) were determined according the methodology published by Ribéreau-Gayon and Stonestreet (1965). All the analytical determinations were realized by duplicate, so the results were the average of two analyses (n = 2).

2.3 Grape volatile compounds extraction and identification

Before analysis, the grapes were defrosted and after the manually extraction of seeds, flesh and skin were blended at room temperature using an Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Thus, 100 grapes per repetition were mixed in the presence of 0.13 M NaF and 50 mg/L ascorbic acid. The triturate was centrifuged at 4,500 rpm for 15 min at 10°C to separate the must from the skins, followed by a filtration through filter paper. The must (ca. 70-80 mL) was percolated through two LiChrolut EN (Merck, Darmstadt, Germany) (100 mg) resin beds (previously preconditioned with 5 mL of dichloromethane (LiChrosolv quality, Merck), 5 mL of methanol, and 5 mL of Milli-Q water, Millipore, U.S.). The column was washed with 10 mL of water, and then with 10 mL of a pentane: dichloromethane (2:1 v/v) mixture. The retained precursors were finally eluted with 10 mL of an ethyl acetate:methanol (9:1 v/v) mixture (ethyl acetate extract). The ethyl acetate extracts were mixed and evaporated under vacuum in a rotary evaporator to 1 mL, and then taken to dryness under gentle nitrogen current. The dry extract was reconstituted in 10 mL of hydrolysis solution (0.2 M citric acid buffer solution at pH 2.5). Acid hydrolysis (100 °C, 4 h) and extraction of the volatiles released was carried out under the conditions described by Loscos et al. (2007) with some modifications. 10 mL of the hidrolized was percolated through a 50 mg LiChrolut EN resin cartridge (previously conditioned with 6 mL dichloromethane, 2 mL methanol, and 6 mL of citric acid buffer solution at pH 2.5). Then, the column was rinsed with 1 mL of water. The precursors were eluted with 700 µL of a dichloromethane. 14 µL of an internal standard solution (4-hydroxy-4-methyl-2-pentanone, and 2-octanol, at a concentration of 450, and 500 µg/g, respectively, in dichloromethane) was added to the eluted sample. Then, the solvent was concentrated under vacuum in a rotary evaporator to 100 µL under gentle nitrogen current. The extract was then analyzed by gas chromatography (GC) detection. Chromatographic analysis was carried out in a HP-6890 equipped with a ZB-Wax plus column (60 m x 0.25 mm x 0.25 µm) from Phenomenex (Phenomenex, Torrance, CA, USA). The column temperature initially was set at 40°C and maintained this temperature for 5 min, then raised to 240°C at a rate of 2°C/min and then maintained 30 min at this temperature. The carrier gas was helium that was fluxed at rate of 3 mL/min. The injection was in split mode 1:25 (injection volume 4 µL), with a flame-ionization-detector (FID detector).

In addition, Kovats retention indices (KI) were calculated for the GC peaks corresponding to identify substance by the interpolation of the retention time of normal alkane (C8 – C20) by

Fluka Buchs (Merck, Darmstadt, Germany), analysed under the same chromatographic conditions. The calculated KI were compared with those reported in the literature for the same stationary phase by comparison of the volatile compounds retention times with those from pure standards.

In the study, 28 aromatic compounds were analyzed although only 14 were detected. The 14 compounds not detected, had not been quantify in all the samples and thus, they are not included in the statistical data.

2.4 Grape skin and seed compounds extraction

Sample of 200 of the berries stored at −20 °C were counted and weighed, and the skins and seeds were separated from the flesh while kept on ice. The skins were manually separated from the berry flesh of the frozen berries, rinsed with distilled-deionized water and extracted at 50°C with 75 rpm stirring for 2 h with a 90% ethanol, 10% water and 5 g/L tartaric acid hydroalcoholic solution (1:10 skin/solvent). In order to minimize proanthocyanidin oxidation, solutions were sparged with nitrogen and the extraction was carried out in the dark. The extracts were crystal-wood filtered and then lyophilized to a dry powder. Analytical determinations for each extract were performed in duplicate, which were then averaged to obtain a value to work with later.

The grape skin parameters determined in the current study were the color intensity (CI); and Total Polyphenol Index (TPI) determinated by Glories method (1978). The Puissant-León method (Blouin, 1992) was used for the determination of total anthocyanins. Total tannin concentration was estimated according to Ribéreau-Gayon and Stonestreet (1966). The extraction methodology was described by Ribéreau-Gayon et al. (2006) and the proanthocyanidin mean degree of polymerization (mDP) were analysed using the methodology described by Kennedy and Jones (2001). Then, according to Kennedy and Jones (2001) methodology, the crude proanthocyanidins were purified using Toyopearl TSK HW 40-F size exclusion media (Tosoh, Japan), packed in an Omnifit column (250 x 25 mm) that was equilibrated with 1:1 MeOH/water containing 0.1% v/v trifluoroacetic acid. The proanthocyanidin powder was dissolved in a minimum amount of this mobile phase and then applied to the column. Then in order to remove carbohydrate and low-molecular-weight flavan-3-ol monomer material, the column was rinsed with five column volumes of the mobile phase. The proanthocyanidins were then eluted with three column volumes of 2:1 acetone/water containing 0.1 % v/v trifluoroacetic acid. The eluent was concentrated under reduced pressure at 35°C to remove acetone, and then lyophilized to a dry powder.

Seeds were manually separated from the berry flesh, rinsed with distilled-deionized water, dried and weighted. A 3 g sample was horizontally placed in a Falcon tube with 50 mL 2:1 acetone/water for maceration during 24 hours at room temperature and 75 rpm stirring. The eluent was concentrated under reduced pressure at 35°C to remove acetone, and then lyophilized to a dry powder. The seed parameters determined in the current study were Total Polyphenol Index (TPI), and total tannin concentration and were performed according to the above-mentioned methods for the grape skins parameters.

In order to determine the tannin main degree polymeration (mDP) estimation in skin and seeds, similar methodoly as Kennedy and Jones, (2001) and García-Esparza et al. (2018) was followed: a 5 mg sample of the dry powder with the proanthocyanidin of interest was reacted in a solution of 0.1 N HCl in MeOH, containing 50 g/L phloroglucinol and 10 g/L ascorbic acid at 50°C for 20 min, and then combined with 2 volumes of 80 mM aqueous sodium acetate to stop the reaction.

The calculation of the apparent mDP consists of the sum of all subunits (flavan-3-ol monomer and phloroglucinol adduct, in moles) divided by the sum of all flavan-3-ol monomers (in moles). Thus, phloroglucinol adducts were analyzed by a reversed-phase HPLC JASCO MD-2010 Plus diode array detector (JASCO, Tokyo, Japan), equipped with a degasser, a quaternary gradient pump, an automatic injector and a thermal stable compartment for the column and a diode array detector (195 to 600 nm), following the methodology proposed by García-Esparza et al. (2018). A LC-Net II/ADC hardware interface between the system components and PC was also used (JASCO, Tokyo, Japan). The chromatographic column was a Gemini NX (particle size 5 μm, 250 x 4.6 mm) purchased from Phenomenex (Torrance, CA, USA). A binary gradient with mobile phases containing 1% v/v aqueous acetic acid (mobile phase A) and 100% MeOH (mobile phase B) was used. Eluting peaks were monitored at 280 nm. According to the methodology following by Kennedy and Jones (2001), the elution conditions were 1.0 mL/min; 5% B for 10 min, a linear gradient from 5 to 20% B in 20 min, a linear gradient from 20 to 40% B in 25 min. The column was then washed with 90% B for 10 min and re-equilibrated with 5% B for 5 min before the next injection.

Galloylation percent (% G) was calculated by dividing the total galloylated proantocyanadin by all identified proantocyanadins and multiplied by 100. Besides, the average molecular weight (aMW) was estimated by the response factor relative to (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin-3-o-gallate (Extrasynthese, Lyon Nord, France).

2.5 Statistical analysis

The quantitative data were analyzed by two-way analysis of variance (ANOVA) with irrigation treatment and year as factors. When the differences were statistically significant at 95 % probability level, ($p < 0.05$), Duncan multiple range tests were performed. Simple linear regression analysis was performed to explore relationships between parameters, and significance levels of the correlation coefficient at 5 % or higher are reported per each studied season. All the stadistical analysis were carried out using SPSS software (SPSS Inc., Chicago, IL) for Windows, Version 11.5. Regression analysis was performed by SigmaPlot 14.0 (Systat Software Inc., San José, CA, USA). Correlation coefficient between variables were calculated in by Pearson's correlation analysis, and *p*-values were acquired to present the significances of the linear fittings.

3. Results and discussion

3.1 Oenological parameters of wine samples

The results for the physico-chemical parameters of the wines for 2012 and 2014 vintages are shown in Table 1. Data of alcohol, pH and the acidity of the wines elaborated at 2013 season are not reported because innacurate values were recorded that vintage due to failure in the analytical equipment employed. In terms of wine alcohol in 2012 (the dry season), the highest wine alcohol concentration was reported in rainfed wines. This was related to the ripeness of the grapes, since at harvest time berries from the rainfed treatment were more ripe, with a higher accumulation of total soluble solids than irrigation treatments (Pérez-Álvarez et al., 2020). Similarly, in 2014, the alcohol values incremented with the two most restrictive water availability treatments (Rainfed and DI) respect to full irrigation (FI) samples. Thus, the alcoholic content of the wines showed a general trend to decrease with water application (Fig. 1A, correlation r^2 = 0.85 and 0.61, in 2012 and 2014, respectively).

In relation to all the wine acids parameters determined, the interaction between year and treatment was not significant (Table 3). Wine pH was not affected by the irrigation regime (Table 3 and Fig. 1B) despite the pH in the grapes of the rainfed treatment was lower than those of the FI ones (Pérez-Álvarez et al., 2020). Contrary to our results, Salón et al. (2005) reported an increase of 0.1 to 0.2 pH units when irrigation was applied. This increase could be decisive for the dose of metabishulphite to be applied and for the risk of sulfur aromas in the wine. Total acidity had higher values in Rainfed wines respect to the FI ones, with intermediate values in those of DI wines. Although in malic, lactic and citric acids content there were not significant differences, in Table 1 can be observed that the malic acid concentration had a trend to increase when water irrigation increased, similar as observed by Salón et al. (2005) in their Bobal wines. These authors suggested that the increase of malic acid under water application compared to

rainfed cultivation is due to a higher rate of degradation in water-stressed vines as a consequence of less shading of the clusters by leaves.

The different effects of irrigation on the main organic acids was also observed by Intrigliolo et al. (2012) in Tempranillo and by Vilanova et al. (2019b) in Verdejo cultivars. However, Romero et al. (2013) reported that titatrable acidity, malic and tartaric acids in wine of Monastrell were not altered by the irrigation treatments imposed. In a Bobal cultivar study, Salón et al. (2005) observed that both, irrigation and seasonal conditions influenced total acidity, being the highest total acidity content in the highest irrigation treatment in the wettest season but, in the dry season, total acidity was higher in the rainfed treatment. In a Cabernet Sauvignon deficit irrigated vineyard, Keller et al. (2008) found the highest wine total acidity values (and lowest pH) in the season with the coolest ripening period of all seasons, and lowest total acidity values (and highest pH) in the year with the warmest ripening period. However, Cancela et al. (2016) reported a significant influence on the alcohol content and on the tartaric and malic acids values, without significant interations between treatment and season.

Regarding all the wines color parameters reported in Table 1, the interation between treatment and year season was not significant indicating that the effect of irrigation application was consistent among seasons. The Rainfed treatment shown the highest values of color intensity (CI), total polyphenol index (TPI) and anthocyanins content, being the lowest values those from FI samples. Thus, the content of those phenolic and color parameters in wines decreased with increasing water application (Fig. 1C-E). Similar as Salón et al. (2005) for Bobal wines, these parameters were also significantly correlated with the water stress integral (which expresses the intensity and duration of stress and was calculated from stem water potential determinations (see Pérez-Álvarez et al., 2020 for more details)) (Fig. 2A-C). Salón et al. (2005) and Vilanova et al. (2019a,b) also observed the effect of the irrigation treatmens on the TPI values. However, regarding the tono of the wines, the effect of the season was more important than the water regime effects being only higher the tono of the FI wines in 2012 respect to these from Rainfed treatment. The reported effects of irrigation on wine phenolic and colour composition might be due to a dilution effect (higher skin-to-pulp-ratio) because of the larger berry size in the irrigated treatments or a direct effect on the concetration of skin phenolic composition. In our case, rainfed and DI treatments presented a higher skin weight percentage *versus* total berry weight (12.4% and 11.7%, respectively, in 2012 and 26.4% (Rainfed) and 26.5% (DI), in 2014) than irrigated grapes (9.6% and 23.3% in 2012 and 2014, respectively) (Table 2), even though the berry size was higher in FI (Pérez-Álvarez et al., 2020). In fact, authors such as Ojeda et al. (2002) and Roby et al. (2004) reported that, in general, water decifit treatments, increase the skin-to-pulp ratio compared to the well-watered wines, increasing the level of skin tannins and anthocyanins. Petrie et al. (2004) suggested that the reduction of water application reduced pericarp mass, which may have

increased the seed-to-pulp ratio and increased the concentration of the phenolic substances in the samples. For its part, Romero et al. (2010, 2013) reported that the increase in wine polyphenol content (tannins and other phenolic compounds) observed under the regulated deficit irrigation (RDI) treatment probably is due to the greater cluster exposure that this water regime provokes. Besides, Romero et al. (2013) suggested that also the phenology timing from which the water stress is applied can affect the RDI effects. They observed that most of the enological and chromatic parameters measured at the end of alcoholic and malolactic fermentation in their Monastrell wines under a RDI strategy which applied mild water stress during the early season (from budburst to fruit set) and a moderate water stress during pre and pos veraison improved wine quality (color intensity, alcohol content, total anthocyanins and total polyphenol index) compared to those wines from RDI applied from veraison to harvest.

3.2 Grape skins and seeds evaluation

In order to determine and explain the reported wine composition effects, direct determinations of skin and seed phenolics were carried out in Tables 2 and 3. As aforementioned, in 2012 and 2014, water restriction treatments showed significant increases in the percentage of skin weight to total berry weight compared to the FI treatment (Table 2). This will affect the content of anthocyanins and other compounds and aromatic precursors found mainly in the skins of the berries. Also the percentage of seed weight *versus* total berry weight was higher with the Rainfed treatment *versus* the irrigated ones, being the total seed weight in the berries of the DI treatment the lowest in 2012 and with intermediate value among the other treatments in 2014 (Table 3). This could affect the tannic compounds, mostly presented in the seeds, and related, among other propierties, to the sensation of astringency of the wines. These data may corroborate those presented by Junquera et al. (2012), who showed that fresh weight is the component of yield most influenced by water restrictions. In 2013, differences between irrigation regime were not significant (possibly due to the aforementioned fact of being a wetter year that minimized the differences between irrigation and Rainfed treatments) (Tables 2 and 3). On the other hand, in 2013 and 2014, the weight of berries (Pérez-Álvarez et al., 2020), seed weight (Table 3) and skin weight percentage to total berry weight (Table 2) were greater than these values in 2012 (year with the driest summer, see more details in Pérez-Álvarez et al., 2020). This matches with the high influence of the year factor found by García-Esparza et al. (2018) over the skin weight of their Cabernet Sauvignon grapes.

Regarding the phenolic compounds, the skin grape anthocyanins and tannins, the total grape anthocyanins content, the grape skin total polyphenol index (TPI) and color intensity (CI) parameters, follow the same pattern; the Rainfed treatment had the highest concentration, being

progressively reduced when irrigation was increasing (Table 2, Fig 1G-I). These results are in agreement with those of Esteban et al. (2001), Kennedy et al. (2002) and Ojeda et al. (2001, 2002) that reported that moderate water deficit increases the phenolic compounds. However, those studies suggested that these desirable effects of DI on grape phenolics occur mainly due to its effect on berry size by selectively increasing the absolute mass of skin tissue (Casassa et al., 2015) rather than a direct biosynthetic effect (Matthews et al., 2006). In the present trial, we reported a direct increase in the concentration of phenolic compounds in the grape skins suggesting that water deficit promoted phenolic biosynthesis Thus, in 2012, the percentage of anthocyanin extractability (% AE) was higher in the water restriction samples with respect to the FI ones. However, in 2013, it was higher in the irrigated samples than in Rainfed (Pérez-Álvarez et al., 2020), indicating that in 2012 the extractability of anthocyanins was higher in the FI treatment and in 2013 in Rainfed with respect to the others treatments. By contrast, the reduction of anthocyanins with the FI treatment was higher respect to the water stress treatments in the three studied seasons.

Other authors attributed the positive impact of mild water deficit to changes in berry skin-to-pulp ratio (Santesteban et al., 2011) or modifications in grape microclimate (Romero et al., 2010). Also in line with our results, Koundouras et al. (2009), Holt et al. (2010) and Cassasa et al. (2015) in their Cabernet Sauvingon studies and Bindon et al. (2011) and Bucchetti et al. (2011) observed that water deficit increased the concentration of skin anthocyanins. Phenolic compounds synthesis is subject to a greater variation than that experienced by other grape compounds, since both, the edaphoclimatic and cultivation conditions of each year influence its formation (Pérez-Álvarez, 2017). This could be related to the fact that the grapevines, especially the berries, synthesize the phenolic compounds via the phenyl-propanoid biosynthetic pathway (Chassy et al., 2012), as defence against adverse situations, either a biotic stress (such as response to a fungus attack) or abiotic stress such as that produced by water stress, UV radiation or temperature variations (Deloire et al., 1998; 1999, Cohen and Kennedy, 2010). It has been hypothesized that pre-veraison RDI may increase anthocyanin concentration by selectively decreasing mesocarp rather than skin growth (Roby et al., 2004; Petrie et al., 2004), or conversely, by selectively increasing the absolute mas of skin tissue (Matthews and Kredemann, 2006). Thus, Kyraleou et al. (2016), observed in a Syrah vineyard in Greece, that with water limitation the berry skin anthocyanins significantly increased, but these differences were maximum 2-3 weeks after veraison and drecreased thereafter to reach similar levels at harvest. Matthews et al. (1990) and Nadal and Arola (1995) showed that, applying water deficits of 70% of the grapevine irrigation needs between veraison and harvest, the anthocyanins production increased, implying an improvement of color in red varieties.

Cassasa et al. (2015) reported that the RDI regimes affected both the concentration (amount per unit fresh weight) and the absolute content (amount per berry) of skin and seed phenolics. Also in an experiment carried out in pots with the Shiraz variety, Ojeda et al. (2002) showed that moderate water deficits increase the grape phenolic compounds biosynthesis and concentration. However, authors as Kennedy et al. (2002) and Bonada et al. (2015) observed that the effects of water availability on the accumulation of tannins in berries are fewer and inconsistent. Thus, in our study, the total grape tannins content was only higher in 2012 in Rainfed than in the other treatments and the skin grape tannins content was higher in Rainfed and DI treatments than in the well wattered samples (Table 2). These results are in agreement with those observed by Intrigliolo et al. (2016), where the final tannin concentration in their Cabernet Sauvignon samples was greater for non-irrigated treatments.

In the case of the grape seed phenolic composition, Rainfed samples presented higher seed tannin content than the irrigation treatments even if grape seed TPI and 100 grapes seed weight were higher from plants irrigated than from those non-irrigated (Table 3). Similarly to our results, Casassa et al (2015) reported increased values of seed tannins over their continuous water deficit Cabernet Sauvignon grapes. They suggested that the higher seed tannin concentration observed in their full deficit treatment compared to the other RDI treatments, was partially due to the lower berry weight. In our case, berries from FI treatment presented lower seed weight percentage *versus* total berry weight than berries under water restriction treatments (Table 3), even though the berry size was higher in FI (Pérez-Álvarez et al., 2020). Pastor del Río and Kennedy, (2006) reported that seed tannin concentration is determined by seed weight and the number of seeds per berry. Thus, Cassasa et al. (2015) suggested that while a severe water deficit might have limited seed tannin biosynthesis (Holt et al., 2010), the simultaneous impact of the deficit on lowering berry size overrides this effect, thereby increasing overall seed tannin concentration. However, Koundouras et al. (2009), and Roby et al. (2004) and Bonada et al. (2015) observed that the water deficit did not alter the tannins of the Cabernet Sauvignon and Shiraz grape seeds, respectively, in spite of its impact on berry weight. For its part, Kyraleou et al. (2017) and Bonada et al. (2015), reported that the decreased seed total tannins content observed under their non irrigated and deficit irrigated conditions, was related to the increased of temperature observed on the berries of those treatments. Bonada et al. (2015) suggested that the heating of grapes reduced tannins by 20% compared to those under ambient conditions. Besides, Kennedy et al. (2000) observed that the amount of seed flavan-3-ols (subunit that conform the tannins) at harvest in Carbenet Sauvignon disminished with the water limitation, while Chacón et al. (2009) reported that, these compounds increased in Merlot seed with the magnitude of water deficiency. On the other hand, Genebra et al. (2000) observed that although several genes of the

biosynthetic pathway of flavan-3-ols were up-regulated, the levels of Tempranillo seed tannins did not shown effect by the irrigation treatments.

3.3 Concentration of skin and seed polymeric proanthocyanidins

The total condensate tannins, analizated by the proanthocyanidin mean degree of polymerization (mDP), the galloylation percentage (% G) and the average molecular weight (aMW) of grape skin and seed tannin are shown in Table 4. As aforementioned, seed tannins consist of only procyanidins, whereas skin tannins include procyanidins and prodelphinidins (Pascual et al., 2016). Thus, as it can be observed in the results (Table 4), seed tannins are shorter, with a lower mDP, while skin tannins are generally larger, with a higher mDP (Chira et al., 2009; Bordiga et al., 2011; Pascual et al., 2016). Therefore, the mDP of skin tannins values were higher than those of seeds and are perceived as astringent in the finished wine (Harrison, 2018), unlike the seed tannins which contribute to wine bitterness (VanderWeide et al., 2020).

Water regimes did not affect the seed polymeric concentration values, while in the grape skin, mDP and aMW values were higher in FI samples than in Rainfed, and also the grape skin % G was lower with the Rainfed treatment. Therefore, it seems that the water deficit regime decreased the polimerization of tanins and probably reduced wine astringency with respect to the FI treatment. According to García-Esparza et al. (2018), the astringency of the wine is related, among others factors, with the tannin mDP of the grapes. Therefore, high grape mDP values and a higher percentage of galloylation (Vidal et al., 2003; Chira et al., 2011) might result in more astringent wines. Thus, Chira et al. (2009) reported that polymeric compounds are increasingly reactive with proteins with increasing mDP, as occurs in Kyraleou et al. (2017) study, where their non-irrigated Syrah grapes presented higher astringency which higher mDP in the polymeric skin fraction, than grapes from deficit irrigated and fully irrigated vines. However, Ojeda et al. (2002) found in their study carried out in Syrah, that the mDP was increased by water deficit treatment, and suggested that berry dehydration could possibly affect the sensorial quality of the wine by diminishing its astringency. For its part, Quijada-Morín et al. (2012) observed that astringency was more affected by the subunit composition of the tannins than by the total concentration or the mDP.

The percentage of grape skin galloylationincreased with water application even in 2013, the year with more rainfall (Table 4). However, in their study with Syrah grapes grown under semiarid conditions in the North of Greece, Kyraleou et al. (2017) found that the grape skin %G values had low consistence throughout the experiment. Sivilotti et al. (2020) neither found remarkable difference between irrigation treatments on the seed structural characteristics of tannins (mDP and % galloylation). Nevertheless, Kyraleou et al. (2017) found higher percentage

of galloylation for seed at harvest in their non-irrigated vines than in the deficit irrigate and fully irrigated samples for both, the oligomeric and polymeric tannins fractions.

Generally, the seed tannins are more astringent than skin tannins because they have a greater degree of galloylation (as ocurrs in our work, Table 4) althought, the bitter and the astringent perception of tannins are affected by their interactions with the soluble polysaccharides present in the grape must (Gil et al., 2012). Also Kyraleou et al. (2017) in Syrah, Chira et al. (2009) in Cabernet Sauvigon and Merlot, Curko et al. (2014) in Plavac mali and Babic, and Rinaldi et al. (2014) in Aglianico cultivars observed higher average values of percentage of gallylation in seed than in skins. The lower proportion of galloylated subunits and the presence of prodelphinidins may be the reason why skin tannins are traditionally regarded in the wine panel as pleasanter and softer and less bitter and astringent than seed tannins (Vidal et al., 2003; Lisjak et al., 2020). According to the regression analysis showed by Kyraleou et al. (2017), a highly significant correlation exits between galloylation percentage (%G) and mDP for both, skins and seeds. They observed that for skin tannins, %G > 2.5, was associated with tannin monomers and oligomers (mDP < 4). By contrast, when mDP is higher than 8 (as occurs in our Bobal grapes independiently of the irrigation treatments, Table 4), it could be associated with an absence of epicatechin-3-O-gallate (ECG) subunits in skin tannins. These authors also observed a similar trend in seeds, with high %G (superior to 6) associated only with monomers, dimers, and trimers, while larger molecules (mDP > 6), presented a lower percentage of galloylation (%G < 5). They argued that larger tannins of both skins and seeds are associated with a low percentage of ECG subunits.

3.4 Grape aroma compounds

Mean values (μg/kg grape) for the aromatic compounds found in the Bobal variety grapes under different irrigation strategies studied throughout 2012, 2013 and 2014 seasons, are shown in Table 5. The analytical method used to extract them allowed us to analyzed 28 compounds although only 14 were identified and quantified in the Bobal grapes including benzenes, volatile phenols, C13 norisiprenoids, lactones, vainillin derivatives and acids chemical families. On the other hand, the major aroma compounds determinated during the analysis in grapes were benzanoic acid (but not in grapes under the highest irrigation dose, FI), 4-vinylphenol, syringaldehyde and octanoic acid (Table 5).

The interaction between both factors, the irrigation treatments and the season, was not significant in any of the determined compounds indicating that the effect of the water regime treatments is maintained during the years of study. The content of volatile compounds such as benzaldehyde, guaiacol, 4-ethylphenol, 4-vinylphenol, α-ionone, γ-decalactone, syringaldehyde,

564 and vainillin increased whith irrigation applications when compared with the Rainfed vines. In
565 any case, the correlation between water application and the benzaldehyde, guaiacol and α-ionone
566 content was significant in the three seasons (Fig. 3A-C, respectively). However, benzanoic acid,
567 3-hydroxybenzaldehyde and octanoic acid increased with DI or Rainfed treatments respect to the
568 highest dose of irrigation (FI). Meanwhile, the content of 2-phenylethanol and isobutyric acid
569 decreased with the DI strategy respect to the other two treatments (Table 5). Thus, according to
570 Alem et al. (2019), water stress impacts on aroma compounds´ biosynthesis in different ways
571 depending on the molecule family concerned. In general, water deficiency affected positively on
572 the abundance of enzymes involved in aroma precursors production (Deluc et al., 2009; Alem et
573 al., 2019).

574 Therefore, in the case of benzene compounds, an important group in the grape varietal
575 aroma which includes aromatic alcohols, aldehydes and volatile phenols (Gómez García-
576 Carpintero et al., 2014), the influence exerted by the irrigation treatments on Bobal grapes was
577 diverse. Benzenoids derivates tend to be synthetized later during grape development and are
578 present in small quantities in grapes (González-Barreiro et al., 2015). Thus, the grape
579 benzaldehyde content, that could add a synergic effect to wine aroma with fruity and floral notes
580 (Gómez García-Carpintero et al., 2011), was higher during the three vintages in those grapevines
581 that had unlimited irrigation (FI) (Table 5). The wettest conditions in 2013, could be the reason
582 why grapes presented this year a trend to had more benzaldehyde than the other seasons. This
583 trend to vary with the availability of water makes that benzaldehyde, compound which possesses
584 a bitter-almond-like odor characteristic of certain wines as those produced from Gramay grapes,
585 acts as marker of the *Botrytis* infection, as well as other compounds as acetic acid, furfural and
586 terpinen-4-ol (Fedrizzi et al., 2011). However, Ju et al. (2018) observed that the accumulation of
587 volatile compounds after RDI treatments was closely related to the amino acids concentration.
588 They reported that the increased of benzaldehyde in Cabernet Sauvigon grapes was closely related
589 to the concentration of leucine, an amino acid which content increased with two deficit irrigation
590 (70% and 80% ETc) treatments compared to full irrigation (100% ETc) samples. On the other
591 hand, in the present research, the 2-phenylethanol content in grapes (aromatic alcohol with rose
592 aroma) was reduced with the DI treatment respect to the others irrigation regime which could
593 have an impact on the "floral" notes of grapes. While one of the precursors in grapes of this
594 aromatic alcohol is the phenylethyl-α-D-glucopyranoside (García et al., 2003), in wines is formed
595 by the catabolism of the amino acid phenylalanine along the alcoholic fermentation process (Bell
596 and Henschke, 2005). Contrary to our findings in which the grapes from DI treatment had an
597 intermediate ºBrix content at harvest respect to grapes from the others treatments (Pérez-Álvarez
598 et al., 2020), Fang and Qian (2012) reported that the synthesis of benzyl alcohol and 2-
599 phenylethanol considerably increased along ripening. However, the content of grape benzanoic

600 acid and 3-hydroxybenzaldehyde was higher in grapes from grapevines with restricted water
601 availability (rainfed and DI) than those of FI treatment. The reduction of the level of benzanoic
602 acid in berries from FI grapevines respect to those of Rainfed, was of the order of 17.44%, 17.55%
603 and 17.51% for each season, respectively.

604 In relation to the volatile phenols, a significant effect of irrigation was observed, guaiacol
605 and 4-vinylphenol had the same pattern; grapes irrigated presented higher values than the Rainfed
606 grapes; 4 ethylphenol was the highest with the DI strategy. In 2013, the 4-ethylphenol and 4-
607 vinylphenol content, was significantly lower than the values of the other seasons. Volatile phenols
608 play an important role in wine aroma, although their influence on the final product may be positive
609 or negative depending on their concentrations (Gómez García-Carpintero et al., 2011). However,
610 as the enzyme that catalyses its formation is inhibited by catechins and catechin tannins, abundant
611 in red wines, the levels of volatile phenols formed in red wines are generally much lower than
612 those in white and rosé wines, although the contents in hydroxycinnamic precursors in the
613 corresponding red musts are higher (Chatonnet et al., 1993). Thus, alike other red wines that
614 contain mostly very low levels of vinylphenols, in our samples the 4-vinylphenol content is higher
615 than 4-ethylphenols, as observed Vilanova et al. (2013) in their young white wines and Siero-
616 Sampedro et al. (2020) in their young red wines of Mencía variety.

617 Regarding α-ionone, a C-13 norisoprenoid which is related to tobacco flavour, an
618 increment was observed in all the seasons on the FI grapes respect to the other two treatments.
619 This could be related to the fact that carotenoids, from whose biodegradation derived the
620 norisoprenoids, are mainly located in the grape skin, whose weight was higher in the FI treatment,
621 even if the ratio % skin weight/total berry weight was lower, than in the water deficit treatments
622 (Table 2). Also Savoi et al. (2016) observed a higher degradation of carotenoids of white grapes
623 under water deficit. By contrast, authors as Deluc et al. (2009), Song et al. (2012) and Savoi et al.
624 (2016, 2017) reported that, in general, water deficit can increase the concentration of C13-
625 norisoprenoides by modulating structural and regulatory genes involves in the biosynthesis of
626 volatile compounds. Sasaki et al. (2016) reported that exposing grapes to light is considerably
627 essential for the biosynthesis of cetain norisoprenoids such as linalool, β-ionone and β-
628 damascenone, which is not consistent with the α-ionone concentration of our trial. Ou et al. (2018)
629 observed that the concentration of β-ionone in their Merlot wines did not differ among irrigation
630 treatments in any of the three studied years. In a Cabertnet Sauvignon assay in which stressed
631 plant received 66% of the water received by the control ones due to a partial root zone drying
632 system, Bindon et al. (2007) observed that the concentration of three important C13
633 norisoprenoids (β-damascenone, β-ionone, and 1,1,6-trimethyl-1,2-dihydronaphthalene)
634 increased by water stress treatments over the two seasons studied. Nevertherles, when results were
635 expressed in terms of ng/berry instead of concentration (ng/g), these authors did not find

636 significant differences between treatments. Thus, they reported that possibly, the differences
637 observed in the concentration results, were due to changes in the volume and/or grape weight as
638 a consequence of water limitation. Also Koundouras et al. (2009) and Alem et al. (2019)
639 suggested that the higher concentration of aroma molecules in grapes is in occasions due to a the
640 reduction of berry size induced by water stress.

641 Among lactones, pantolactone (2,4-dihydroxy-3,3-dimethylbutyric acid-γ-lactone)
642 content did not shown differences between seasons and treatments. In the case of the γ –
643 decalactone, the most irrigated grapes (FI) had the highest content, especially in 2012, the driest
644 year (Table 5). Lactones are a special subgroup of esters formed by internal esterification between
645 carboxyl and hydroxyl groups of the parent molecule. Most lactones in wine appear to be
646 produced during fermentation, although its origin is also lies in grapes, contributing to the varietal
647 aroma (Ribéreau-Gayon et al., 2006). They are apparently derived from amino or organic acids,
648 notably glutamic and succinic acids.

649 Table 5 shows the increases in vanillin and syringaldehyde content with the irrigation
650 treatments, however, vainillin content was highest with the DI treatment and syringaldehyde with
651 FI respect to those grapes of the other two treatments. Both phenolic aldehydes compounds,
652 vanillin and syringaldehyde, possess vanilla-like fragrances. In the family of the vainillin
653 derivatives, there are compounds whose presence in the wine in large quantities is due to their
654 extraction from the wood during aging, being much smaller compared to the amounts released by
655 hydrolysis of their glycosidic precursors. However, in wines without aging, the reserve of
656 aromatic potential of the precursors can have a subtle influence on the aroma and flavor of the
657 wines.

658 The content of the fatty acids determined in the grape samples (isobutyric and octanoic
659 acids) varied in different way with the irrigation treatment; isobutyric acid increased with the
660 Rainfed treatment and also with the maximum irrigation dose, however, the isobutyric octanoic
661 acid increased in the treatments where less water content was applied. Also Gómez García-
662 Carpintero et al. (2011) found the isobutyric acid and octanoic acid between the most abundant
663 acids in their Bobal wines. Fatty acids are found in berries esterified in the form of phospholipids,
664 neutral lipids and glycolipids (Serrano de la Hoz, 2014). It is in the grape skins where most of the
665 fatty acids are found, being its content between 1.5 and 3 times higher than in the pulp (Bayonove,
666 2003). Fatty acids have been described with fruity, cheesy, fatty, and rancid notes (Rocha,
667 Rodrigues, Coutinho, Delgadillo, and Coimbra, 2004). Deluc et al. (2009) observed that water
668 deficit affected, among others, fatty acid metabolic pathways and, althought they did not provide
669 aroma precursors analysis showed that water deficiency, impacted positively on the abundance of
670 enzymes involved in aroma precursors production (Alem et al., 2019).

671 On the other hand, Hernández-Orte et al. (2015) shown that the vintage introduced
672 significant differences in most of the compounds tested in their work, being most of the precursors
673 synthesis in warmer years and under more sun-exposed grapes in Tempranillo, Merlot and
674 Gewurztraminer varieties. As aforementioned, in our case, the vintage only affected some of the
675 aromatic compounds (volatile phenols) detected in Bobal grapes. However, in their white grape
676 varieties studied, Bouzas-Cid et al. (2018a,b) and Vilanova et al. (2019b), reported that the
677 volatile organic compounds were more influenced by the inter-annual variation than by the in-
678 season variation due to irrigation treatments. Bouzas-Cid et al. (2018a) also reported that mild or
679 moderate levels of water deficit result in limited or no effects on must, wine composition and
680 wine sensory features.

681 In general, as results in this study, irrigation strategies could regulate the grape aroma
682 content respect to the Rainfed and the non-water limit treatments. Thus, an increase in certain
683 grape aroma precursors can be obtained by reducing the water content used in the vineyard.
684 However, due to a) the complexity of the formation of volatile compounds in grapes, which are
685 determined by the variety and may be influenced by vineyard management and biotic or abiotic
686 stresses (Alem et al., 2019), b) the differential responses of specific metabolic pathways that these
687 compounds present in grapes, and c) how little studied is the Bobal variety despite its optimum
688 winemaking qualities for producing quality wines, among other factors, additional studies are
689 needed to improve our understanding how the modifications in grape arompatic potential will
690 affect the final wines tasting attributes and scores.

691

692 **4. Conclusions**

693 This study demonstrates the important role that irrigation regimes has on grapevine cv.
694 Bobal wine composition and grape quality parameters. For grapes harvested at given similar
695 moment, wines from rainfed or deficit irrigated vines were more concentrated in terms of alcohol
696 and phenolic composition resulting in much higher colour content. This was not only due to a
697 dilution effect due to the positive effects of irrigation on berry weight but also because higher
698 concentration of phenolic compounds in seed and particularly skin tissues. The percentage of the
699 skin and seed weight compared to the total weight of the grapes, as well as the skin anthocyanins
700 content and seed and skin tannins were higher in those treatments less irrigated. The degree of
701 polymerization (mDP, aMW) of the skin tannins and the percentage of galloylation (%G) were
702 lower in the Rainfed or even in DI grapes respect to FI ones. This lead to think that the perception
703 of astringency and possibly the sensation of bitternes (since the seed bitterness can be
704 compensated by the milder bitterness of the skin tannins) of wines from Bobal grapes under
705 Rainfed and DI regime will be lower than those of the FI grapevines.

In addition, it was also demonstrated that the irrigation regime influences the grape aroma precursors and therefore determining the final wine sensory attributes. However, water deficit affects aroma compounds´ biosynthesis in different ways depending on the molecule family concerned. Thus, grapes from deficit irrigation strategy were richer than those with Rainfed treatment in volatile phenols content, which plays an important role in wine aroma, although their influence on the final product may be positive or negative depending on their concentrations. Also grapes from deficit irrigation regime had higher benzanoic acid, 3-hydroxybenzaldehyde and octanoic acid concentration than grapes from unlimited water supply grapevines, which could be attributed to a change caused by the water deficit in the metabolic pathways of these groups of compounds. Since the aroma precursors will mark the sensory attributes of the wine, it has been seen that, possibly, a correct management of the water treatment in the plant, can shape the profiles of the chemical families that the consumer will find in the wine. From a practical point of view, it can be concluded that watering at 35% of the ETc, is a recommended irrigation strategy for optimizing grape skin, seed and volatile composition in comparison with full irrigation allowing to increase yield in comparisons to rainfed vines as reported in our companion study by Pérez-Álvarez et al. (2020).

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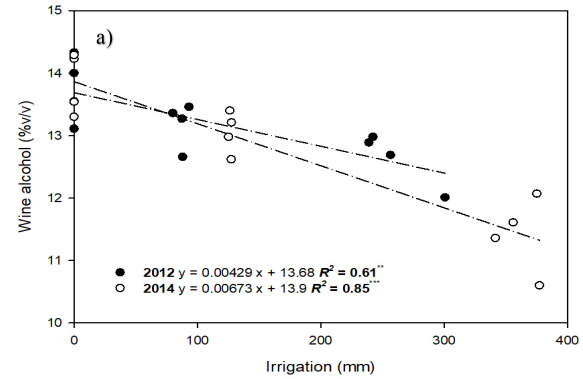
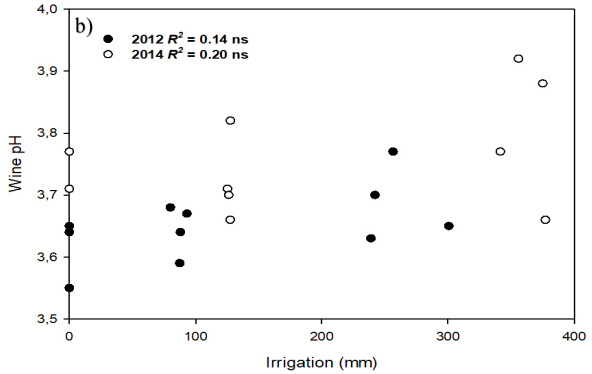
Figure captions

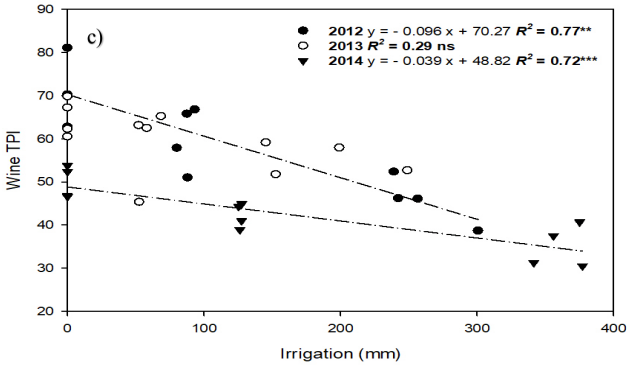
Figure 1. Relationship of irrigation (mm) and (A) alcoholic content (%vol/vol), (B) pH, (C) total polyphenol index, (D) anthocyanins (mg/l) (, (E) color intensity in Bobal wines and (F) 100 grapes weight (g), (G) grape skin anthocyanins (mg/g), (H) grape skin total polyphenol index and (I) grape skin color intensity of the 2012, 2013 and 2013 vintages. Lines of linear regression and values of the coefficient of determination (R^2) with indication of significance at p <0.001 (***), p = 0.05-0.001 (**), p < 0.05 (*) or non significant (ns) are shown.

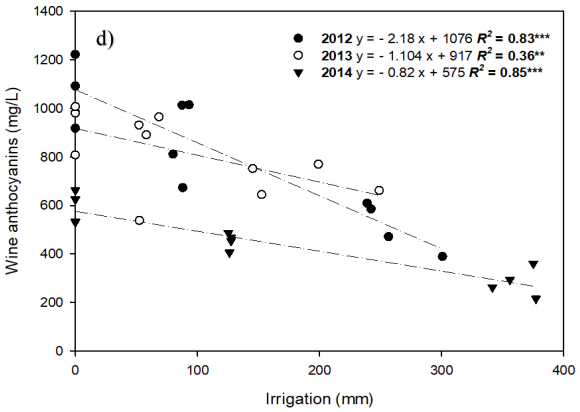
Figure 2. Relationship of the water stress integral (MPa*year) calculated from stem water potential measured at mid-day and (A) total polyphenol index, anthocyanin content (mg/L) (B), and color intensity (C) in Bobal wines of the 2012, 2013 and 2013 vintages. Lines of linear regression and values of the coefficient of determination (R^2) with indication of significance at p <0.001 (***), p = 0.05-0.001 (**), p < 0.05 (*) or non significant (ns) are shown.

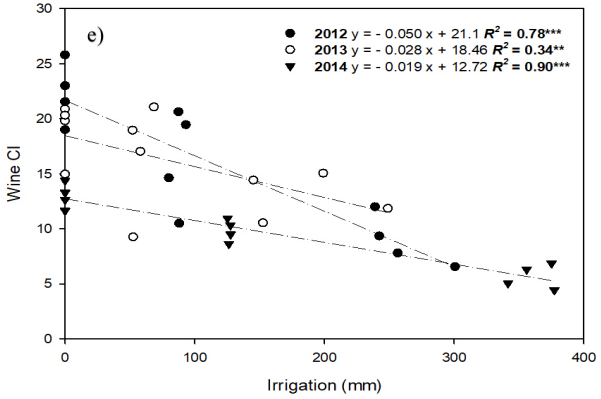
Figure 3. Relationship of irrigation (mm) and (A) benzaldehyde, (B) guaiacol and (C) α-ionone content (μg/kg of grape) in Bobal grapes of the 2012, 2013 and 2013 vintages. Lines of linear regression, when significant, and values of the coefficient of determination (R^2) with indication of significance at p <0.001 (***), p = 0.05-0.001 (**), p = 0.05 (*) or non significant (ns) are shown.

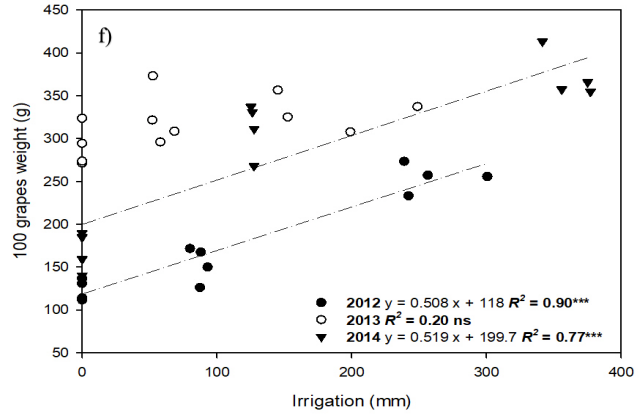

a)
Wine alcohol (%v/v)
Irrigation (mm)
2012 y = 0.00429 x + 13.68 R² = 0.61**
2014 y = 0.00673 x + 13.9 R² = 0.85***

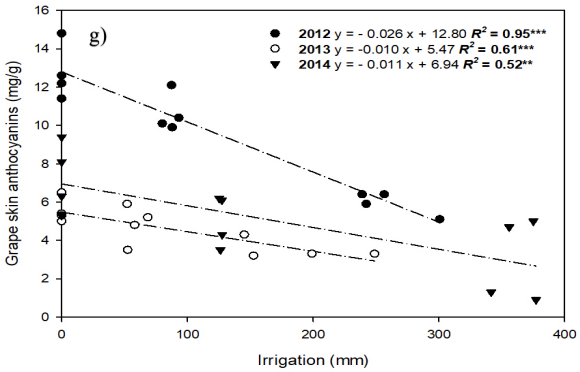

b)
Wine pH
Irrigation (mm)
2012 R² = 0.14 ns
2014 R² = 0.20 ns

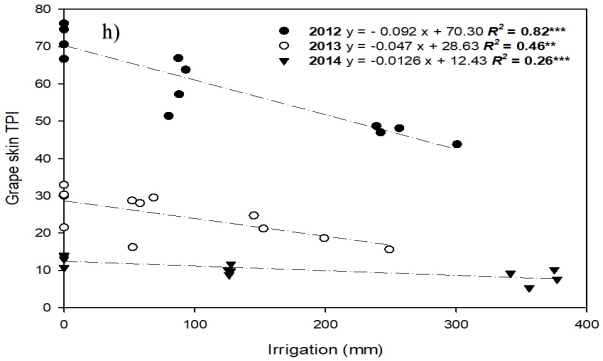

c)
Wine TPI
Irrigation (mm)
2012 y = - 0.096 x + 70.27 R² = 0.77**
2013 R² = 0.29 ns
2014 y = - 0.039 x + 48.82 R² = 0.72***


d)
Wine anthocyanins (mg/L)
Irrigation (mm)
2012 y = - 2.18 x + 1076 R² = 0.83***
2013 y = - 1.104 x + 917 R² = 0.36**
2014 y = - 0.82 x + 575 R² = 0.85***


e)
Wine CI
Irrigation (mm)
2012 y = - 0.050 x + 21.1 R² = 0.78***
2013 y = - 0.028 x + 18.46 R² = 0.34**
2014 y = - 0.019 x + 12.72 R² = 0.90***


f)
100 grapes weight (g)
Irrigation (mm)
2012 y = 0.508 x + 118 R² = 0.90***
2013 R² = 0.20 ns
2014 y = 0.519 x + 199.7 R² = 0.77***


g)
Grape skin anthocyanins (mg/g)
Irrigation (mm)
2012 y = - 0.026 x + 12.80 R² = 0.95***
2013 y = -0.010 x + 5.47 R² = 0.61***
2014 y = - 0.011 x + 6.94 R² = 0.52**


h)
Grape skin TPI
Irrigation (mm)
2012 y = - 0.092 x + 70.30 R² = 0.82***
2013 y = -0.047 x + 28.63 R² = 0.46**
2014 y = -0.0126 x + 12.43 R² = 0.26***

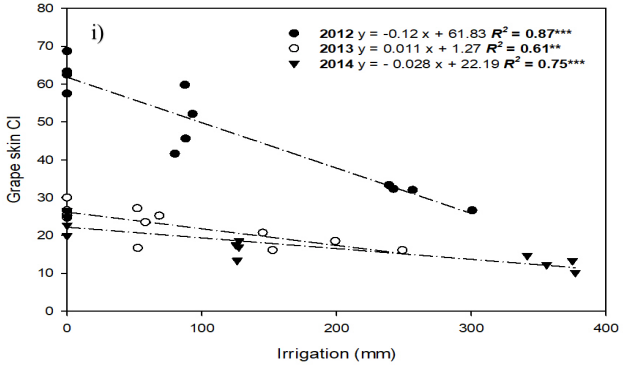

i)
Grape skin CI
Irrigation (mm)
2012 y = -0.12 x + 61.83 R² = 0.87***
2013 y = 0.011 x + 1.27 R² = 0.61**
2014 y = - 0.028 x + 22.19 R² = 0.75***

Figure 1.

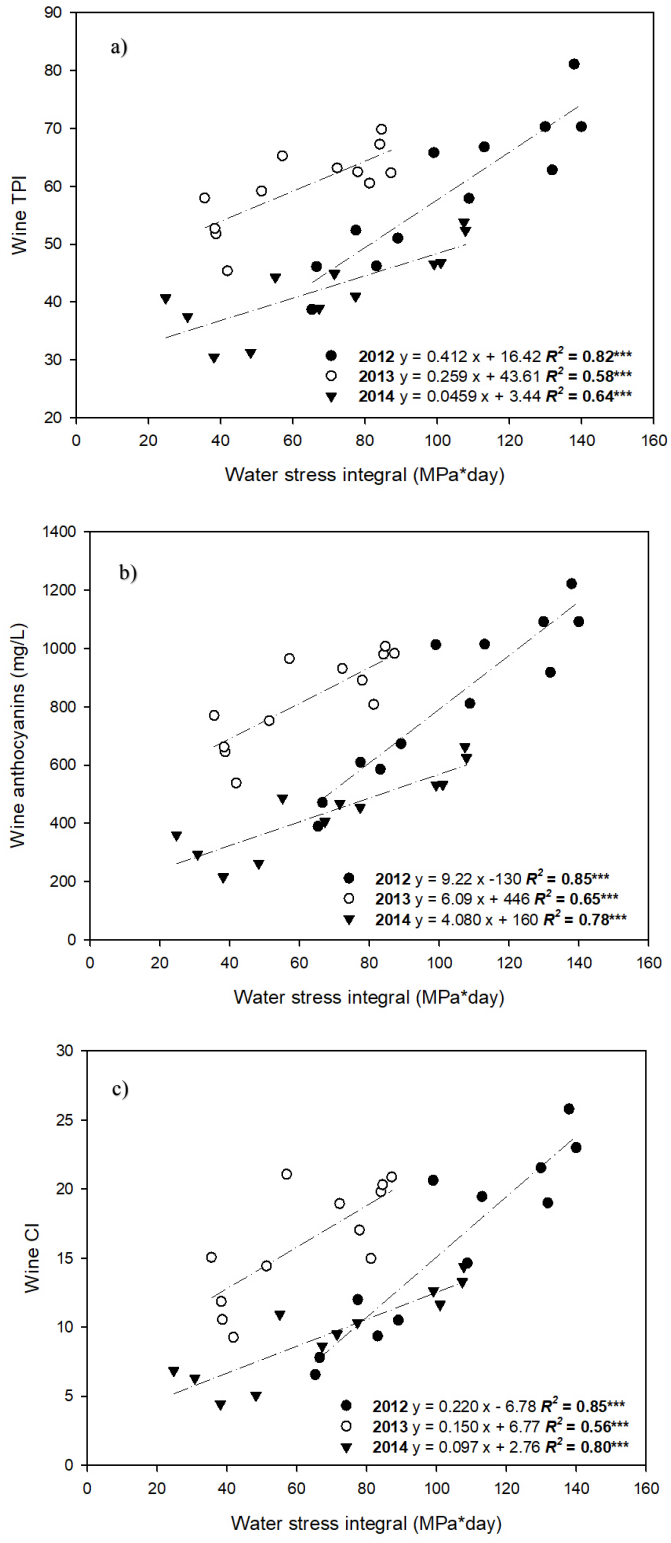

a)
Wine TPI
Water stress integral (MPa*day)
2012 y = 0.412 x + 16.42 R2 = 0.82***
2013 y = 0.259 x + 43.61 R2 = 0.58***
2014 y = 0.0459 x + 3.44 R2 = 0.64***
b)
Wine anthocyanins (mg/L)
Water stress integral (MPa*day)
2012 y = 9.22 x -130 R2 = 0.85***
2013 y = 6.09 x + 446 R2 = 0.65***
2014 y = 4.080 x + 160 R2 = 0.78***
c)
Wine CI
Water stress integral (MPa*day)
2012 y = 0.220 x - 6.78 R2 = 0.85***
2013 y = 0.150 x + 6.77 R2 = 0.56***
2014 y = 0.097 x + 2.76 R2 = 0.80***

Figure 2.

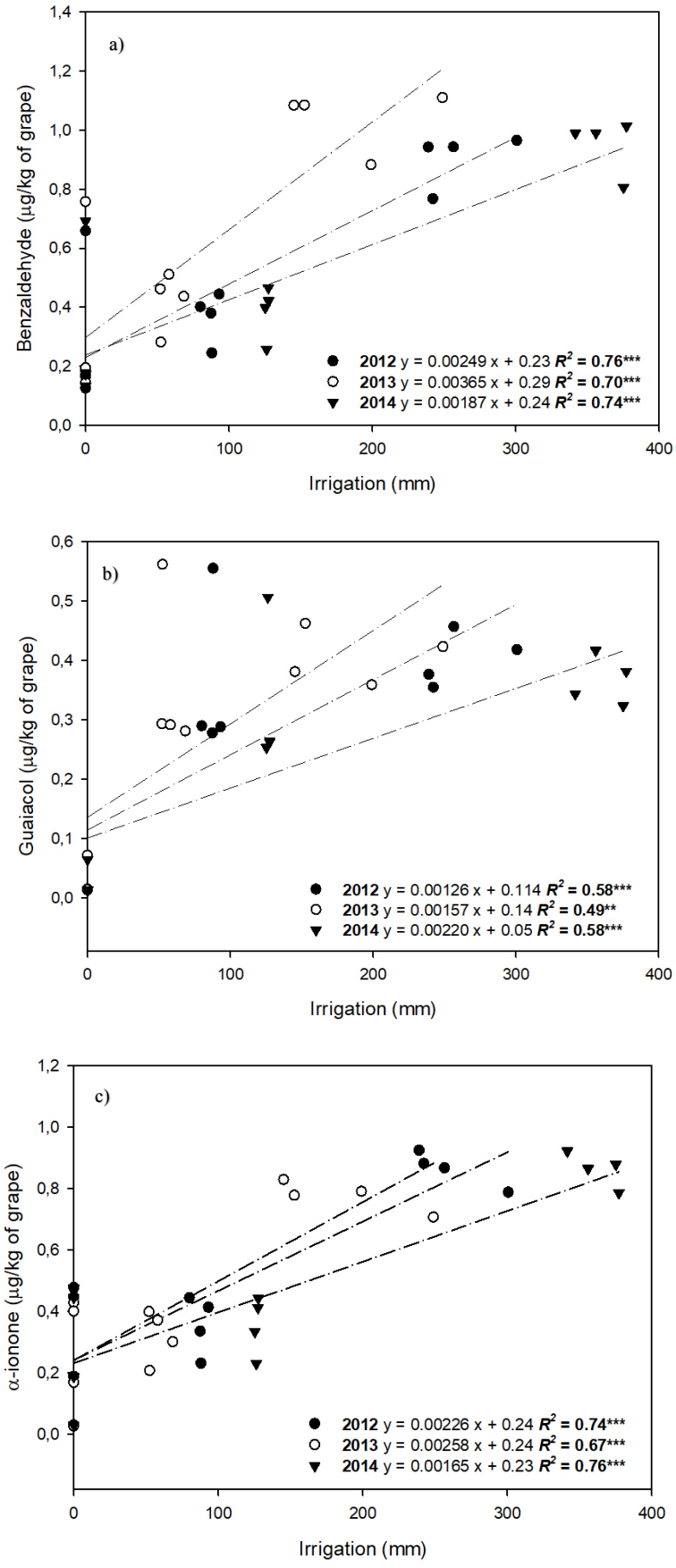

a)
Benzaldehyde (µg/kg of grape)
Irrigation (mm)
2012 y = 0.00249 x + 0.23 R² = 0.76***
2013 y = 0.00365 x + 0.29 R² = 0.70***
2014 y = 0.00187 x + 0.24 R² = 0.74***
b)
Guaiacol (µg/kg of grape)
Irrigation (mm)
2012 y = 0.00126 x + 0.114 R² = 0.58***
2013 y = 0.00157 x + 0.14 R² = 0.49**
2014 y = 0.00220 x + 0.05 R² = 0.58***
c)
α-ionone (µg/kg of grape)
Irrigation (mm)
2012 y = 0.00226 x + 0.24 R² = 0.74***
2013 y = 0.00258 x + 0.24 R² = 0.67***
2014 y = 0.00165 x + 0.23 R² = 0.76***

Figure 3.

Tables

Table 1. Wine Bobal enological parameters elaborated from grapes of each treatment (Rainfed; DI: irrigated at 35% of the ETc and FI: full irrigation, 100% of the ETc) during the three seasons of the study (2012, 2013 and 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 treatment	Year (p-value)	T x year (p-value)
Alcohol (% v/v)	**Rainfed**	13.75b	-	13.84b	13.79c	**	**
	DI	13.18ab	-	13.05b	13.12b		
	FI	12.64a	-	11.41a	12.03a		
pH	**Rainfed**	3.63a	-	3.72a	3.68a	***	ns
	DI	3.64a	-	3.72a	3.68a		
	FI	3.69a	-	3.80a	3.75a		
Total acidity (g/L)	**Rainfed**	6.34a	-	6.28b	6.31b	ns	ns
	DI	6.14a	-	6.15b	6.15ab		
	FI	6.11a	-	5.75a	5.93a		
Malic acid (g/L)	**Rainfed**	1.66a	-	1.36a	1.51a	ns	ns
	DI	1.61a	-	1.70a	1.66a		
	FI	1.95a	-	1.97a	1.96a		
Lactic acid (g/L)	**Rainfed**	0.53a	-	1.01a	0.68a	***	ns
	DI	0.68a	-	0.82a	0.75a		
	FI	0.51a	-	0.85a	0.68a		
Citric acid (g/L)	**Rainfed**	0.34a	-	0.21a	0.28a	***	ns
	DI	0.33a	-	0.23a	0.28a		
	FI	0.32a	-	0.21a	0.27a		
CI^+	**Rainfed**	22.33c	18.99a	12.98c	18.10c	***	ns
	DI	16.30b	16.57a	9.84b	14.24b		
	FI	8.92a	12.96a	5.65a	9.18a		
TPI	**Rainfed**	71.12b	64.98a	49.90c	62.00c	***	ns
	DI	60.37b	59.06a	42.27b	53.90b		
	FI	45.85a	55.40a	35.00a	45.42a		
Anthocyanins (mg/L)	**Rainfed**	1081.0b	944.5a	588.0c	871.17c	***	ns
	DI	877.7b	831.2a	453.7b	720.92b		
	FI	513.5a	707.2a	283.2a	501.33a		

Tono	**Rainfed**	0.43a	0.38a	0.50a	0.44a	***	ns
	DI	0.45ab	0.40a	0.51a	0.45a		
	FI	0.48b	0.41a	0.59a	0.49a		

For each parameter and year, different letters indicate significant differences between treatments at 95% (p <0.05) based on Ducan multiple range test. The probability levels used were $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and ns, not significant. [+]Abbreviations: CI: color intensity, TPI: total polyphenol index.

Table 2. Parameters of total grape skin phenolic composition at harvest for Bobal grapes 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 (2012-2014). 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
100 grapes weight (g)	**Rainfed**	123.22a	290.66a	168.40a	194.09a	***	***
	DI	153.71b	324.76a	311.76b	263.41b		
	FI	254.87c	331.66b	372.74c	319.76c		
% skin weight/grape weight	**Rainfed**	12.36b	22.20a	26.39b	20.31a	***	ns
	DI	11.68b	22.46a	26.46b	20.20a		
	FI	9.59a	21.65a	23.31a	18.18a		
Skin grape anthocyanins (mg/g skin)	**Rainfed**	12.74c	5.57b	7.29b	8.53c	***	**
	DI	10.61b	4.85b	5.02ab	6.83b		
	FI	5.94a	3.53a	2.97a	4.15a		
Total grape anthocyanins (mg/g grape)	**Rainfed**	1.58c	1.23b	1.93b	1.58c	ns	ns
	DI	1.24b	1.0a9b	1.31ab	1.28b		
	FI	0.57a	0.76a	0.70a	0.68a		
Skin grape tannins (mg/g skin)	**Rainfed**	25.18c	13.84b	9.14a	16.05c	***	ns
	DI	20.18b	13.46b	8.97a	14.20b		
	FI	16.91a	10.41a	8.39a	11.90a		
Total grape tannins (mg/g grape)	**Rainfed**	3.11b	3.08a	2.41a	2.87b	**	ns
	DI	2.35a	3.03a	2.34a	2.57ab		
	FI	2.02a	2.81a	2.45a	2.43a		
Grape skin TPI[+]	**Rainfed**	72.04c	28.66b	12.89b	37.86c	***	***
	DI	59.84b	25.60ab	10.11a	31.85b		
	FI	46.89a	20.02a	8.08a	25.00a		
Grape skin CI	**Rainfed**	62.97c	26.71b	23.47c	37.72c	***	***
	DI	49.75b	23.14b	16.57b	29.82b		
	FI	31.04a	17.85a	12.62a	20.50a		

For each parameter and year, different letters indicate significant differences between treatments at 95% ($p < 0.05$) based on Ducan multiple range test. The probability levels used were $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and ns, not significant. [+] Abbreviations: TPI, Total polyphenol index; CI, color index.

Table 3. Parameters of total grape seed phenolic composition at harvest for Bobal grapes in the rainfed application and in the treatments waterd at 35 (DI) and 100% (FI) of the estimated crop evapotranspiration (ETc) during each studied season (2012-2014). 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
% grapes seed weight/grape weight	**Rainfed**	3.49c	2.45a	3.37b	3.10c	ns	***
	DI	2.57b	2.39a	2.54a	2.50b		
	FI	2.00a	2.27a	2.31a	2.20a		
Tannins(mg/g seed)	**Rainfed**	101.78a	97.75a	93.29a	97.61b	ns	ns
	DI	105.05a	71.90a	66.43a	81.13ab		
	FI	83.89a	75.32a	62.28a	73.83a		
Tannins (mg/g grape)	**Rainfed**	3.53b	2.36a	3.15b	3.01b	ns	ns
	DI	2.74ab	1.72a	1.69a	2.05a		
	FI	1.65a	2.00a	1.44a	1.70a		
Grape seed TPI[+]	**Rainfed**	21.50a	28.78a	25.52a	25.26a	ns	ns
	DI	32.97b	34.69a	41.58b	36.20b		
	FI	32.31b	37.10a	39.39ab	35.82b		

For each parameter and year, different letters indicate significant differences between treatments at 95% (p <0.05) based on Ducan multiple range test. The probability levels used were $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and ns, not significant. [+] Abbreviations: TPI: total polyphenol index.

Table 4. Concentration of skin and seed polymeric proanthocyanidins for Bobal grapes 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 (2012-2014). 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
			SKIN				
Grape skin mDP+	**Rainfed**	13.64a	15.53a	13.31a	14.16a	***	**
	DI	14.75a	16.37ab	14.30a	14.14a		
	FI	10.47a	17.13b	15.84a	14.48a		
Grape skin aMW	**Rainfed**	4096.90a	4546.76a	3935.03a	4192.90a	***	**
	DI	4411.14a	4823.11ab	4246.84a	4493.70a		
	FI	3114.083a	5051.07b	4692.96a	4286.04a		
Grape skin galloylation	**Rainfed**	4.65ab	1.73a	2.61a	3.00a	***	***
(%)	**DI**	5.01b	2.69b	3.62b	3.77c		
	FI	3.68a	2.96c	3.39b	3.34b		
			SEED				
Seed tannins mDP	**Rainfed**	7.12a	7.73a	7.00a	7.29a	ns	ns
	DI	7.01a	7.31a	6.62a	6.98a		
	FI	6.94a	6.65a	7.19a	6.93a		
Grape seed aMW	**Rainfed**	2245.88a	2433.39b	2153.47a	2277.58a	ns	ns
	DI	2211.81a	2299.88ab	2076.43a	2196.04a		
	FI	2195.39a	2090.40a	2261.33a	2182.37a		
Grape seed galloylation	**Rainfed**	16.38a	16.15a	15.64a	16.06a	ns	ns
(%)	**DI**	16.14a	16.04a	16.68a	16.29a		
	FI	17.25b	15.88a	16.84a	16.66a		

For each parameter and year, different letters indicate significant differences between treatments at 95% ($p < 0.05$) based on Ducan multiple range test. The probability levels used were $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and ns, not significant. + Abbreviations: mDP, mean degree of polymerization; aMW, average molecular weight.

Table 5. Mean values (µg/kg of grape) of the aromatic compounds from the Bobal grapes of the treatments (Rainfed; DI: irrigated at 35% of the ETc and FI: full irrigation, 100% of the ETc) throughout the three seasons of the study (2012, 2013 and 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
Benzenes							
Benzaldehyde	**Rainfed**	0.28a	0.32a	0.29a	0.29a	ns	ns
	DI	0.37a	0.42a	0.38a	0.39a		
	FI	0.90b	1.04b	0.95b	0.96b		
2-Phenylethanol	**Rainfed**	0.20a	0.24a	0.22a	0.22b	ns	ns
	DI	0.19a	0.20a	0.20a	0.20a		
	FI	0.18a	0.22a	0.20a	0.20b		
Benzanoic acid	**Rainfed**	7.15b	7.90b	7.18b	7.41c	ns	ns
	DI	3.33ab	3.68ab	3.34ab	3.45b		
	FI	0.41a	0.45a	0.41a	0.42a		
3-Hydroxybenzaldehyde	**Rainfed**	0.34b	0.35b	0.32b	0.34b	ns	ns
	DI	0.43b	0.45b	0.41b	0.43b		
	FI	0.05a	0.05a	0.05a	0.05a		
Volatile phenols							
Guaiacol	**Rainfed**	0.28a	0.28a	0.20a	0.27a	ns	ns
	DI	0.35b	0.36b	0.32b	0.34b		
	FI	0.40b	0.41b	0.37b	0.39b		
4-Ethylphenol	**Rainfed**	0.34a	0.22a	0.22a	0.26a	**	ns
	DI	0.46a	0.30b	0.34a	0.37b		
	FI	0.26a	0.17ab	0.19a	0.21a		
4-Vinylphenol	**Rainfed**	2.46a	1.60a	2.09a	2.05a	**	ns
	DI	6.26b	4.07b	5.32b	5.21b		
	FI	5.30b	3.45b	4.51b	4.42b		
C-13 norisoprenoids							
α Ionone	**Rainfed**	0.28a	0.25a	0.28a	0.27a	ns	ns
	DI	0.35a	0.32a	0.35a	0.34a		
	FI	0.86b	0.78b	0.86b	0.83b		
Lactones							
Pantolactone	**Rainfed**	0.47a	0.42a	0.46a	0.45a	ns	ns
	DI	0.36a	0.32a	0.36a	0.35a		

	FI	0.44a	0.40a	0.44a	0.43a		
γ-decalactone	**Rainfed**	0.21a	0.15a	0.19a	0.18a	ns	ns
	DI	0.28a	0.20a	0.25a	0.24a		
	FI	0.81b	0.57b	0.36b	0.58b		
Vainillin derivatives							
Syringaldehyde	**Rainfed**	2.46a	2.62a	2.37a	2.49a	ns	ns
	DI	3.28a	3.49a	3.16a	3.31a		
	FI	5.83b	6.21b	5.62b	5.89b		
Vainillin	**Rainfed**	0.38a	0.33a	0.37a	0.36a	ns	ns
	DI	0.61a	0.54b	0.60b	0.59b		
	FI	0.42a	0.37a	0.41a	0.40a		
Fatty acids							
Isobutyric acid	**Rainfed**	0.30a	0.29a	0.30a	0.30b	ns	ns
	DI	0.13a	0.13a	0.13a	0.13a		
	FI	0.29a	0.29a	0.30a	0.29b		
Octanoic acid	**Rainfed**	2.37a	2.16a	2.32a	2.28b	ns	ns
	DI	2.75a	2.50a	2.69a	2.65b		
	FI	1.16a	1.06a	1.14a	1.12a		

1167 For each compound and year, different letters indicate significant differences between treatments at 95% (p <0.05) based on Ducan multiple range test. The probability levels
1168 used were $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and ns, not significant.

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