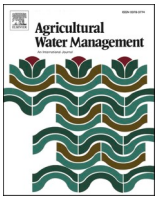
Agricultural Water Management 256 (2021) 107078

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat



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

V. Lizama [a], E.P. Pérez-Álvarez [b,*], D.S. Intrigliolo [b,c], C. Chirivella [d], I. Álvarez [a], M. J. García-Esparza [a]

[a] Universitat Politècnica de València, Instituto de Ingeniería de Alimentos para el Desarrollo, C/ Fausto Elio, 24, 46011 Valencia, Spain
[b] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Departamento de Riego, E-30100, Murcia, Espinardo, Spain
[c] Consejo Superior de Investigaciones Científicas – Centro de Investigación sobre Desertificación (CSIC-UV-GV), 46113 Valencia, Moncada, Spain
[d] Servicio de Producción Ecológica, Innovación y Tecnología, 46340 Valencia, Requena, Spain

ARTICLE INFO

Handling Editor: Dr. B.E. Clothier

Keywords:
Bobal
Regulated deficit irrigation
Tannins
Anthocyanins
Skin and seed polyphenols
Aroma compounds

ABSTRACT

This study aims to determine the effects and the response to irrigation regimes (i) rainfed, (ii) deficit irrigation (DI), and (iii) full irrigation (FI) on wine and grape skin, seed and aromatic composition of grapevine cv. Bobal. The results show that the deficit irrigation treatment can modulate some important parameters of grapes 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 the wines decreased with the application of water, while berry weight increased. Wine colour, total phenolics, and anthocyanins increased when water application was restricted due to 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 with respect to rainfed. Benzanoic acid, 3-hydroxybenzaldehyde and octanoic acid content also increased with respect to the full irrigation treatment. These increases can favour metabolic pathways that enhance specific volatile aromas in the wines, affecting their sensory quality. The overall the results presented demonstrate the important role played by the irrigation regime in modulating Bobal grapes and wine composition.

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's photosynthetic activity, affecting the grapevine vegetative development and the overall performance Koundouras et al. (2009). Therefore, the expected water scarcity in many winegrowing areas as a result of the climate change scenario, may result in negative effects on wine quality such as inhibition of anthocyanin accumulation, changes or losses in grape color and/or acidity, an increase in pH, alcohol degree, aromatic compounds volatilization (through the production of 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). Deficit irrigation (DI) is an irrigation strategy that is widely used in viticulture. It consists on the application of amount of water to substitute only a part of the grapevine potential evapotranspiration (ETc), either during previously-established phenological stages, or during the entire crop cycle (Intrigliolo and Castel, 2010).

Soil, climate and agronomic management implemented on the vineyard are closely linked to the fruit morphological development, which affects 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 (present in the pulp cells). From a winemaking perspective, and according to Mirás-Avalos et al. (2019), the high

* Corresponding author.
E-mail address: evapilar.perez@icvv.es (E.P. Pérez-Álvarez).

https://doi.org/10.1016/j.agwat.2021.107078
Received 14 December 2020; Received in revised form 10 July 2021; Accepted 13 July 2021
Available online 27 July 2021

surface area to volume ratio of the small berries, makes these smaller grapes preferable over the larger berries. Thus, what should be discerned is, whether the desirable effects of DI on grapes and wine phenolic compounds, are due to an increased concentration (*i.e.*, on a fresh weight basis) or occur due to enhanced biosynthesis (*i.e.*, on a per berry basis)(Casassa et al., 2015). Therefore, factors such as environmental conditions (Koundouras et al., 2006), grape variety (Kallithraka et al., 2006), and viticultural practices (Kyraleou et al., 2015) have influenced the accumulation of plant secondary metabolites, including phenolic compounds in grapes. These compounds are known to play many roles in the plant's response to a large variety biotic and abiotic stresses, with special importance to water stress (Caldwell et al., 2003; Pinasseau et al., 2017).

From both, the chemical and sensory point of view, anthocyanins (glycosylated pigments which provide color), and proanthocyanidins or condensed tannins (mainly responsible for the astringent and bitter properties), are the most decisive phenolic compounds in the qualitative properties of red grapes and wines (Casassa et al., 2015). Also, through their covalent reaction with anthocyanins, tannins modulate the color of wine forming polymeric pigments with astringent properties, and orange or brick-red pigments (Somers, 1971). The intensity of wine astringency has been reported to be linked 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-Morín et al., 2012) have suggested that the astringency of the wines was strongly influenced by the composition of the tannins than by the total content of phenolic compounds, while others reported that the structural composition of the tannins were less correlated with astringency than the total content of phenolics and tannins (Kyraleou et al., 2016). Also, 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 effect of irrigation on the accumulation of anthocyanins in grapes has been more extensively studied than the influence of irrigation on the accumulation of grape proanthocyanidins. In general, as a consequence of the effect of mild water deficit, several authors have reported an increase in anthocyanin content, attributed to changes in berry skin-to-pulp ratio (Santesteban et al., 2011) or modifications in grape microclimate (Romero et al., 2010), and a qualitative modification of the anthocyanin set when more detailed analysis were performed (Castellarin et al., 2007; Castellarin and di Gaspero, 2007; Bucchetti et al., 2011; Ollé et al., 2011; Hochberg et al., 2015). On the other hand, the effect of irrigation management on grape tannins accumulation has not been extensively reported. Likewise, comparing cv. Chardonnay (Deluc et al., 2009) or cv. Syrah (Hochberg et al., 2015) to cv. Cabernet Sauvignon, the specificity variety of these responses has been described. This may be related to differences in their phenological stages or to water use behavior (Hochberg et al., 2015), as the composition of phenolic compounds is influenced differentially by the early or late water deficit events (Ojeda et al., 2002; Ollé et al., 2011; Casassa et al., 2015).

Thus, in general, when grapevines are submitted to water deficit, studies have reported an increase in anthocyanins content and in total polyphenol index (TPI). For instance, in the Kyraleou et al. (2016) study, the concentration of Syrah berry skin anthocyanins increased when water was limited, although they observed that the differences were the highest 2–3 weeks post-veraison and decreased afterwards to similar levels at harvest. Intrigliolo and Castel (2009) observed, in Tempranillo irrigated grapevines, that the effect of irrigation on grape color and anthocyanins was dependent on water stress timing and severity. In this respect, Castellarin et al. (2007) and Castellarin and Di Gaspero (2007) observed that the anthocyanins synthesis pathway is positively affected by water stress. Matthews et al. (1990) and Nadal and Arola (1995), reported low contents of phenolic compounds, anthocyanins and tannins on less stressed cv. Cabernet Sauvignon grapevines due to the effect of a post-veraison water stress. 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 led to a decrease in anthocyanin synthesis, and in cv. Monastrell, Romero et al. (2010) found that the total grape phenolic content was severely affected by intense water stress. Cassasa et al. (2015) observed, over three consecutive growing seasons on Cabernet Sauvignon grapes and wines, that the concentrations of skin and seed phenolics were the most affected by the DI regimes, suggesting that the impact of these techniques is rather indirect and based on a reduction of berry size.

Regarding berry tannins, studies on the impact of water availability are scarce and inconsistent. Tannins are commonly found in skins, seeds and stems, although their nature varies depending on the part of the cluster they are found (Palcual et al., 2016). Seed tannins are composed of oligomers and polymers of three flavan-3-ol subunits: (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-gallate (Prieur et al., 1994), while skin tannins also contain (-)-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 (Palcual et al., 2016). According to previous studies, such as those from Kennedy et al. (2000) and Bonada et al. (2015), the accumulation of tannins in grapes is little affected directly by the water deficit of the vine. However, in Cabernet Sauvignon assays, Kennedy et al. (2000) reported that the seed flavan-3-ols concentration at harvest decreased due to the water stress, while in Merlot seeds, Chacón et al. (2009) found that the content of flavan-3-ols and tannins was enhanced depending on the magnitude of water limitation. Genebra et al. (2014) observed that the content of tannins in Tempranillo seeds did not suffer variations due to the effect of irrigation, although several genes from the biosynthetic pathway of flavan-3-ols were up-regulated, while Zarrouk et al. (2012) reported that irrigation increased the regulation of skin tannins. Roby et al. (2004) and Koundouras et al. (2009) stated 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 Rio and Kennedy (2006) reported that seed tannin concentration was also determined by the weight of the seed and the number of seeds per berry. After analyzing the effect of four DI regimes on Cabernet Sauvignon grapes, Cassasa et al. (2015) suggested that tannin biosynthesis was not altered by DI as there was no effect of any of the deficit irrigation regimes on seed and skin tannin content. They also observed that both, the DI regimes and the growing seasons, had an effect on the amounts of tannins extracted from seeds, whereas the proportion of skin tannins was not affected by either of these factors. Thus, the results of the effect of water stress on grape phenolics have so far been inconsistent in many experimental studies. It is generally unclear if the reported effects were due to berry dehydration, an increased skin to pulp ratio, or a change in compound metabolism (García-Esparza et al., 2018). Also, according to Cassasa et al. (2015), phenolics content was season-dependent, meaning that different growing seasons are linked with specific biosynthetic effects that alter the phenolic content and, eventually, extraction and retention into wine.

On the other hand, the wine aroma is complex and is one of its main organoleptic properties, and it is the final result of a long biological, biochemical, and technological sequence. The wine aroma profile is mainly dependent on two important groups of compounds; the free fraction of the aromas, which are the volatile compounds, and the bound fraction of aromas, which are the aromatic precursors. These volatile compounds are terpenes, C13-norisoprenoids, benzenoids, C6 compounds, and pyrazines, with about 800 volatile substances coming from the grape, and the aromatic precursors, which are non-volatile and odorants, although they are able to release aromas under the influence of various factors (Bayonove, 2003). The aromatic precursors can be

grouped into two groups based 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, with the structure of the precursor being 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 glutathione, glycosidic 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 strongly influenced by a large variety factors, both biotic and abiotic, including environmental factors such sunlight (Zhang et al., 2017), water availability (Bouzas-Cid et al., 2018a, 2018b; Vilanova et al., 2019a, 2019b), and viticultural practices such as cluster thinning (Feng et al., 2017) or plant growth regulator application, such as abscisic acid (ABA) (Jia et al., 2018), jasmonic acid (D'Onofrio et al., 2018), among others.

Therefore, scarce information has been published about the influence of the irrigation management 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 the quality of red and rosé wines. Also, according to these authors, the market acceptance of Bobal wines is primarily based on its high color intensity and tannin concentration. In addition, Sivilotti et al. (2020) suggested 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 applied to a Bobal vineyard for three consecutive vintages in a semi-arid climate, in order to obtain further 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. The vineyard is located near Requena, Valencia, Southeast of Spain (Latitude: 39º 29N; Longitude: 1º 13 W; elevation above sea level: 750 m) and belongs to the Utiel-Requena Denomination of Origin (D.O.). The 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. (2021). Briefly, the vineyard soil was Typic Calciorthid and the climate in the area was continental Mediterranean and semiarid. The mean annual temperature was 13.7, 13.7 and 14.8 ºC, respectively in 2012, 2013 and 2014 seasons, and the annual rainfall was 291, 345 and 272 mm, respectively each year. Also, the reference evapotranspiration (ETo) was 1220, 1212 and 1280 mm in 2012, 2013 and 2014, respectively.

The experiment utilized a randomized block design with three treatments and four replications per each treatment. Each replicate had 35 grapevines spread over five consecutive rows of seven plants each, although only the three inner rows were utilized for sampling, with the two outer rows used as borders. Since planting in 2002, the entire experimental vineyard was irrigated by a deficit irrigated regime with the standard irrigation rates in the area, around 60 mm per season. From 2012 on, 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, through the application of 100% ETc. Irrigation scheduling was carried out weekly using the ETc = ETo x Kc formula, where the crop coefficient (Kc) was 0.6, according to Williams and Ayars (2005) considerations, and ETo was the reference evapotranspiration registred by a meteorological station located nearby. Irrigations began when the rainfed grapevines reached threshold values of stem water potential (Ψstem) of − 0.6 to − 0.7 MPa. For all of the plants, the frequency of irrigation varied from 1 to 2 times per week in spring to 5–6 times per week in mid-summer, with the irrigation doses applied being different in each irrigation event, depending on the corresponding water regime. Thus, grapevines from the DI treatment received 91, 74 and 125 mm of irrigation water in 2012, 2013 and 2014 respectively; meanwhile, FI grapevines received 251, 225, and 375 mm of water, respectively, each season (Pérez-Álvarez et al., 2021). The drip lines had emitters spaced at 1.25 m, which provided 3.5 Lh^{-1} of water to the grapevines. The grapevines were planted at a spacing of 2.5 × 1.4 m, so they had two emitters each.

2.2. Grape sampling, winemaking process and oenological parameter analysis

For each repetition, 20 grapevines were harvested at the optimum time 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. Harvest day was on the 10th, 30th and 29th of September in the 2012, 2013 and 2014 seasons, respectively. Samples consisting of 600 berries were randomly taken from each repetition and the weight of 100 grapes from each repetition was determined. Then, the grapes were divided into two set of 300 berries, one set for determining technological and polyphenolic parameters (see details in Pérez-Álvarez et al. (2021), and another for analysing flesh and seed, and aromatic compounds. Grapes were stored in isothermal containers to be taken to the laboratory, where they were kept at − 20 ºC until analytical determinations.

Following the harvest, the 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 25 ºC in stainless steel containers, one for each repetition. A *Saccharomyces cerevisiae* commercial yeast strain were inoculated and all microvinifications were maintained in skin contact for 7 days, automatically punched every 4 h. The wine probable alcohol degree (% v v^{-1}), pH, total acidity (g L^{-1} tartaric acid), and malic acid (g L^{-1}) were analysed according to the methodology established by the OIV (2003). Lactic and citric acids (g L^{-1}) were also analysed enzymatically (Miura One, Tecnología Difusión Ibérica, Barcelona, Spain). Colour intensity (OIV methodology), and total polyphenol index were analysed according to Ribéreau-Gayon et al. (2000). Wine anthocyanins (mg L^{-1}) were determined according the methodology by Ribéreau-Gayon and Stonestreet (1965). All the analytical determinations were performed in duplicate, with the results being the average of two analyses (n = 2).

2.3. Grape volatile compounds extraction and identification

Before analysis, the grapes were defrosted and after the manual extraction of seeds, flesh and skin, they were blended at room temperature with an Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Thus, 100 grapes per repetition were mixed with 0.13 M NaF and 50 mg L^{-1} ascorbic acid and centrifuged at 4500 rpm and at 10 °C for 15 min to separate the must from the skins, after which it was filtered. Following the protocol by Loscos et al. (2007), about 70–80 mL of must were run through two LiChrolut EN (Merck, Darmstadt, Germany) (100 mg) resin beds which had been previously conditioned with 5 mL each of dichloromethane (LiChrosolv quality, Merck), methanol, and Milli-Q water, Millipore, U.S.). According to the conditions described by Loscos et al. (2007) with some modifications, the acid hydrolysis (100 °C, 4 h) and volatiles extraction was carried out. Briefly, 10 mL of

the hydrolyzed sample was percolated through a 50 mg LiChrolut EN resin cartridge that was preconditioned with 6 mL of dichloromethane, 2 mL of methanol, and 6 mL of citric acid buffer solution at pH 2.5. Afterwards, 1 mL of water was used for rinsed the column. The precursors were eluted with 700 μL of dichloromethane. Afterwards, 14 μL of an internal standard solution was added to the eluted sample. The internal standard solution was 450 μg g^{-1} of 4-hydroxy-4-methyl-2-pentanone. Then, the solvent was concentrated under vacuum in a rotary evaporator down to 100 μL under a gentle nitrogen current. A gas chromatography (GC) detection was used for analyzing the extract. Thus, an HP-6890 chromatograph equipped with a ZB-Wax plus column (60 m × 0.25 mm × 0.25 μm) from Phenomenex (Phenomenex, Torrance, CA, USA) was used. The column temperature was initially set to 40 °C for 5 min, and then raised to 240 °C at a rate of 2 °C min^{-1}, with this temperature maintained for 30 min. Helium was the carrier gas, fluxed at rate of 3 mL min^{-1}. A split mode 1:25 (injection volume 4 μL) injection was used, with a flame-ionization-detector (FID detector).

Volatile compounds were previously identified with a mass spectrometer (Finnigan TRACEMET MS, TermoQuest, Austin, USA) using the same column that was later used in the GC-FID for the quantification of the compounds. Identification of the compounds was carried out by comparison of the mass spectrometric data and chromatographic retention of pure reference compounds: benzaldehyde (Fluka), guaiacol (Aldrich), 4-ethyl phenol(Fluka), 4-viniphenol (Fluka), α-ionone (Aldrich), γ-decalactone (Fluka), syringaldehyde (Aldrich), 2 phenylethanol, benzanoic acid (Fluka), 3-hydroxybenzaldehyde (Aldrich), pantolactone (Aldrich), octanoic acid (Aldrich) and isobutyric acid (Aldrich). The standards were used to make the calibration curves for the quantified volatile compounds.

Besides, to identify the substance through the interpolation of the retention time of normal alkane (C8 – C20), Kovats retention indices (KI) were calculated for the GC peaks with Fluka Buchs (Merck, Darmstadt, Germany), analysed under the same chromatographic conditions. The KI reported in the literature for the same stationary phase were used to calculate the KI by comparing the volatile compounds retention times with those from pure standards.

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

2.4. Grape skin and seed compounds extraction

A sample of 200 of the grapes stored at − 20 °C was counted and weighed, and while on ice, the skins and seeds were manually separated from the flesh. The extraction of the skins were performed at 50 °C with 75 rpm stirring for 2 h in a 5 g L^{-1} tartaric acid hydroalcoholic solution (1:10 skin/solvent), 10% water, 90% ethanol and in the dark. The extracts were filtered though crystal-wool and lyophilized to a dry powder. For the statistical analysis, the analytical determinations were performed in duplicate for each extract.

The grape skin parameters determined in the current study were the color intensity (CI); and Total Polyphenol Index (TPI), which were determined using the Glories (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 the Kennedy and Jones (2001) methodology, to purify the crude proanthocyanidins, a Toyopearl TSK HW 40-F size exclusion media (Tosoh, Japan), which was utilized.

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

In order to determine the skin and seeds tannin main degree polymeration (mDP), a similar methodology as Kennedy and Jones (2001) and García-Esparza et al. (2018) was followed: in a solution of 0.1 N HCl in MeOH, with 50 g L^{-1} phloroglucinol and 10 g L^{-1} ascorbic acid, a 5 mg sample of the dry powder with the proanthocyanidin of interest was reacted at 50 ºC for 20 min. In order to stop the reaction, it was combined with 2 volumes of 80 mM aqueous sodium acetate.

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, the methodology proposed by García-Esparza et al. (2018) was used to analyze the phloroglucinol adducts by a reversed phase HPLC-DAD (JASCO, Tokyo, Japan).

In order to determine the percentage of galloylation percent (% G), the total galloylated proanthocyanidin were divided by all the identified proanthocyanidins and multiplying by 100. Also, to calculate the average molecular weight (aMW), the response factor relative to (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin-3-o-gallate (Extrasynthese, Lyon Nord, France) was used.

2.5. Statistical analysis

Irrigation treatment and year were the factors used for analyzing the quantitative data with a two-way analysis of variance (ANOVA). When the differences were statistically significant at 95% probability level, ($p < 0.05$), Duncan's multiple range tests were performed. To know the relationships between parameters, a simple linear regression analysis was performed. Significance levels of the correlation coefficient at 5% or higher are reported per each studied season. All the statistical analysis were carried out using SPSS software (SPSS Inc., Chicago, IL) for Windows, Version 11.5. The regression analysis was performed with SigmaPlot 14.0 (Systat Software Inc., San José, CA, USA). In order to obtain the significances of the linear fittings, correlation coefficients between variables were calculated with Pearson's correlation analysis, and *p*-values were presented.

3. Results and discussion

3.1. Oenological parameters of wine samples

The results obtained for the physico-chemical parameters of the wines for 2012 and 2014 vintages are shown in Table 1. Data on the alcohol content, pH, and the acidity of the wines elaborated in the 2013 season are not reported because inaccurate values were recorded on that vintage due to failure of the analytical equipment employed. In terms of wine alcohol in 2012 (the driest season with rainfall of 114 mm *vs* 208 and 119 mm from April to 30th September 2013 and 2014, respectively), the highest wine alcohol concentration was reported in rainfed wines. This was related to the ripeness of the grapes, as at harvest time the berries from the rainfed treatment were more ripe, with a higher accumulation of total soluble solids (23.5º Brix), than the irrigation treatments (22.1 and 21.3º Brix, in DI and full irrigation (FI) grapes, respectively) (Pérez-Álvarez et al., 2021). Similarly, in 2014, the alcohol values increased with the two most restrictive water availability treatments (Rainfed and DI) with respect to FI samples. Thus, the alcohol content of the wines showed a general decreasing trend with the application of water (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),

Table 1
Wine Bobal enological parameters measured in grapes from 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 treatment (T), 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	T x Year
Alcohol (% v v^{-1})	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^{-1})	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^{-1})	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^{-1})	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^{-1})	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^{-1})	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		

For each parameter and year, different letters indicate significant differences between treatments at 95% ($p < 0.05$) based on Ducan's 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.

despite the pH being lower in the grapes from the rainfed treatment as compared to those from the FI treatment (Pérez-Álvarez et al., 2021). Contrary to our results, Salón et al. (2005) reported an increase of 0.1–0.2 pH units when irrigation was applied. This increase could be decisive for the dose of metabisulphite to be applied and for the risk of sulfur aromas in the wine. The total acidity values were found to be higher in Rainfed wines with respect to the FI ones, with intermediate values found for the DI wines. Although significant differences were not found for the contents of malic, lactic, and citric acids, Table 1 shows the increasing trend of the malic acid concentration when water irrigation increased, as similarly observed by Salón et al. (2005) in their Bobal wines. These authors suggested that the increase in malic acid with the application of water as compared to rainfed cultivation was 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 reported by Intrigliolo et al. (2012) in Tempranillo, and by Vilanova et al. (2019b) in Verdejo cultivars. However, Romero et al. (2013) reported that titratrable acidity, malic and tartaric acids in Monastrell wine 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, with the total acidity being the highest in the highest irrigation treatment in the wettest season, although in the dry season, the total acidity was higher in the rainfed treatment. In a deficit irrigated Cabernet Sauvignon vineyard, Keller et al. (2008) found the highest total acidity values in wine (and lowest pH) in the season with the coolest ripening period of all the seasons, and the lowest total acidity values (and highest pH) in the year with the warmest ripening period. However, Cancela et al. (2016) reported a significant effect on the content of alcohol and tartaric and malic acids, without significant interactions between treatment and season.

Regarding all the wines color parameters reported in Table 1, the interaction between treatment and year was not significant, indicating that the effect of the irrigation application was consistent between years. The Rainfed treatment showed the highest values of color intensity (CI), total polyphenol index (TPI) and anthocyanins content, with the lowest values being those from the FI samples. Thus, the content of these phenolic compounds and the color parameters in wines decreased with increasing water application (Fig. 1C-E). Similar to Salón et al. (2005) for Bobal wines, these parameters were also significantly correlated with the water stress integral (which expresses the stress duration and intensity and was calculated from stem water potential determinations (see Pérez-Álvarez et al. (2021) for more details)) (Fig. 2A-C). Briefly, according to the results reported by Pérez-Álvarez et al. (2021), the grapevines from the rainfed treatment showed moderate values of stress (Ψstem = −1.2 MPa) in the 2013 and 2014 seasons, and severe stress values (Ψstem = −1.29 to −1.45 MPa) during most of their pre- and post-veraison, and ripening periods (from July 2nd to September 3rd) in 2012. Fully irrigated vines did not experience any water stress during the growing cycle, and in general, the DI plants had an intermediate Ψstem values between the rainfed and FI treatments. Salón et al. (2005) and Vilanova et al. (2019a, 2019b) also observed the effect of the irrigation treatments on the TPI values. The reported effects of irrigation on wine phenolics and colour composition may 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 concentration of skin phenolic composition. In our case, rainfed and DI treatments had 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 berries were larger in size in FI (Pérez-Álvarez et al., 2021). In fact, authors such as Ojeda et al. (2002) and Roby et al. (2004) observed that in general, water deficit treatments, increase the skin-to-pulp ratio as compared to the well-watered wines, increasing the concentrations of skin tannins and anthocyanins. Petrie et al. (2004) suggested that the decrease in water input reduced pericarp mass, which may have increased the seed-to-pulp ratio and increased the concentration of the phenolic substances in the samples. On their part,

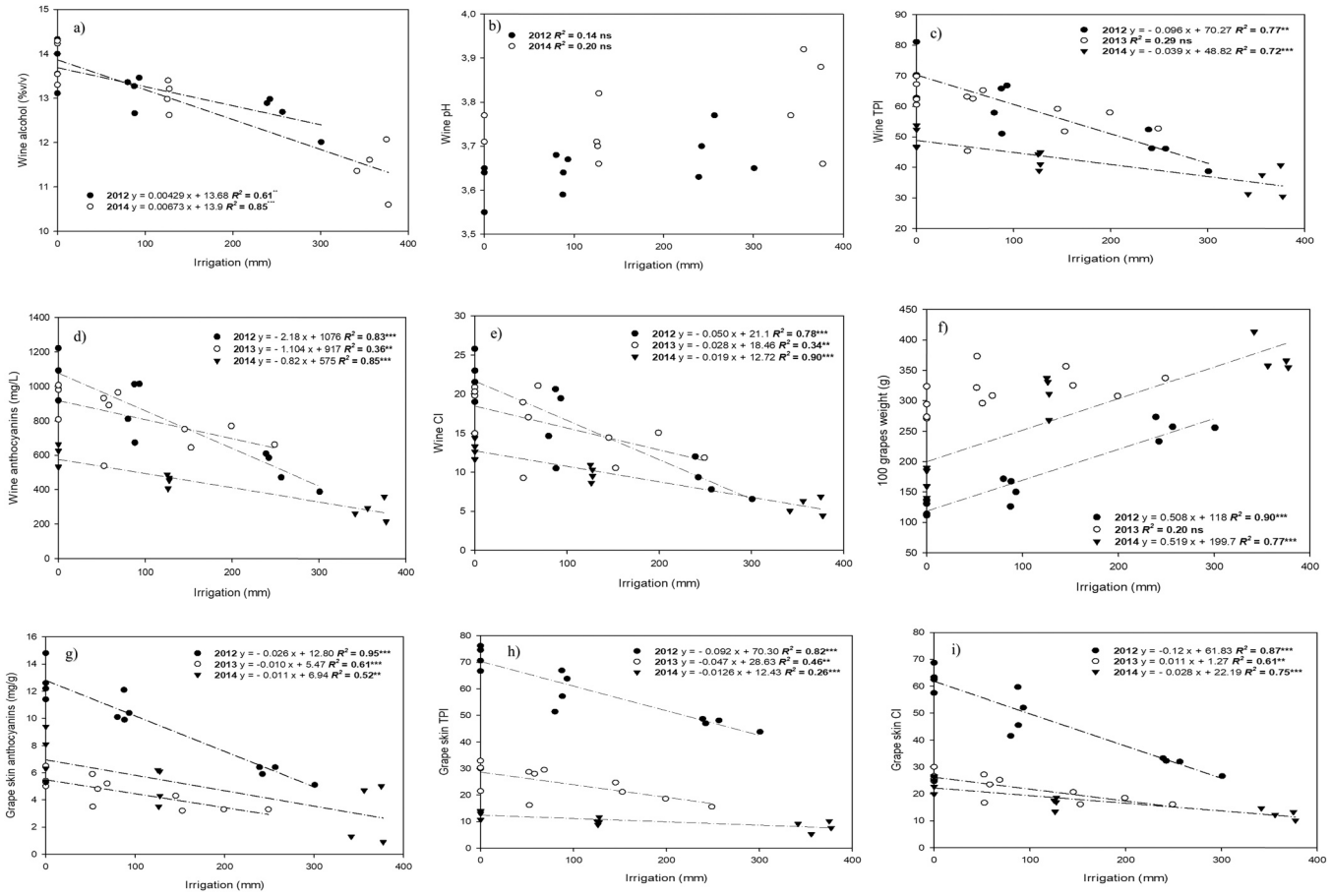


Fig. 1. Relationship of irrigation (mm) and (a) alcoholic content (%vol vol^{-1}), (b) pH, (c) total polyphenol index, (d) anthocyanins (mg L^{-1}) (e) color intensity in Bobal wines and (f) 100 grapes weight (g), (g) grape skin anthocyanins (mg g^{-1}), (h) grape skin total polyphenol index and (i) 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.

Romero et al. (2010, 2013) reported that the rise in wine polyphenol content (tannins and other phenolic compounds) observed under the regulated deficit irrigation (RDI) treatment, was probably due to the greater cluster exposure provoked by this water regime. Also, Romero et al. (2013) also suggested that the phenological period during which the water stress is applied can affect the RDI effects. They observed that most of the chromatic and enological parameters measured at the end of both alcoholic and malolactic fermentation in their Monastrell wines, under an RDI strategy that applied mild water stress from budburst to fruit set (during the early season), and a moderate water stress during pre and post-veraison, improved wine quality (color intensity, alcohol content, total anthocyanins and total polyphenol index) as compared to those wines from the RDI treatment 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 and the results are shown 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 as compared to the FI treatment (Table 2). This affected 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, with the total seed weight in the berries from the DI treatment being the lowest in 2012 and obtaining an intermediate value among the other treatments in 2014 (Table 3). This could affect the tannic compounds, mostly present in the seeds, and related, among other properties, to the sensation of astringency of the wines. These data may corroborate those presented by Junquera et al. (2012), who showed that fresh weight was the most-influenced yield component by water restrictions. In 2013, differences between irrigation regimes were not significant (possibly due to this year being wetter and the rainfall during pre-veraison sufficient to increase the water available for the grapevines, which minimized the differences between the irrigation and Rainfed treatments) (Tables 2 and 3). On the other hand, in 2013 and 2014, the weight of berries (Pérez-Álvarez et al., 2021), seed weight (Table 3), and skin weight percentage to total berry weight (Table 2) were greater than these values from 2012 (year with the driest summer, see more details in Pérez-Álvarez et al. (2021). This matched with the high influence of the year factor found by García-Esparza et al. (2018) on 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, followed the same pattern; the Rainfed treatment had the highest concentration, which progressively decreased when irrigation was enhanced (Table 2, Fig. 1G-I). These results are in agreement with those from Esteban et al. (2001), Kennedy et al. (2002), and Ojeda et al. (2001, 2002), who reported that moderate water deficit increases the concentration of phenolic compounds. However, those studies suggested that these desirable effects of DI on grape phenolics mainly occur due to its effect on berry size by selectively enhancing the absolute mass of skin tissue

Table 2
Parameters of total grape skin phenolic composition at harvest for Bobal grapes in the rainfed treatment 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 treatment (T), 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	Rainfed	12.74c	5.57b	7.29b	8.53c	***	**
(mg g^{-1} skin)	DI	10.61b	4.85b	5.02ab	6.83b		
	FI	5.94a	3.53a	2.97a	4.15a		
Total grape anthocyanins	Rainfed	1.58c	1.23b	1.93b	1.58c	ns	ns
(mg g^{-1} grape)	DI	1.24b	1.0a9b	1.31ab	1.28b		
	FI	0.57a	0.76a	0.70a	0.68a		
Skin grape tannins (mg g^{-1} 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	Rainfed	3.11b	3.08a	2.41a	2.87b	**	ns
(mg g^{-1} grape)	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's 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 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 treatment (T), 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	Rainfed	3.49c	2.45a	3.37b	3.10c	ns	***
weight/grape weight	DI	2.57b	2.39a	2.54a	2.50b		
	FI	2.00a	2.27a	2.31a	2.20a		
Tannins (mg g^{-1} 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^{-1} 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's 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.

(Casassa et al., 2015) rather than a direct biosynthetic effect (Matthews and Kriedemann, 2006). In this trial, we reported a direct increase in the concentration of phenolic compounds in the grape skins, suggesting that water deficit promoted phenolic compound biosynthesis. Thus, in 2012, the percentage of anthocyanin extractability (% AE) was higher in the water restricted samples with respect to the FI ones. However, in 2013, it was higher in the irrigated samples than in the Rainfed one (Pérez-Álvarez et al., 2021), indicating that in 2012 the extractability of anthocyanins was higher in the FI treatment, and in 2013 in the Rainfed treatment with respect to the other treatments. In contrast, the reduction of anthocyanins with the FI treatment was higher with respect to the water stress treatments in the three studied seasons.

Other authors have 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 Sauvignon studies, and Bindon and Kennedy (2011) and Bucchetti et al. (2011), reported that the concentration of skin anthocyanins was enhanced by the water deficit regime. Phenolic compounds synthesis is subject to a greater variation than that experienced by other grape compounds, as both the edapho-climatic 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 phenylpropanoid biosynthetic pathway (Chassy et al., 2012), as a defence against adverse situations, either a biotic stress (such as response to a fungus attack), or a 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 anthocyanin concentration may be increased by the selective decrease in the mesocarp rather than the skin growth due to a pre-veraison RDI treatment (Roby et al., 2004; Petrie et al., 2004), or *vice versa*, by

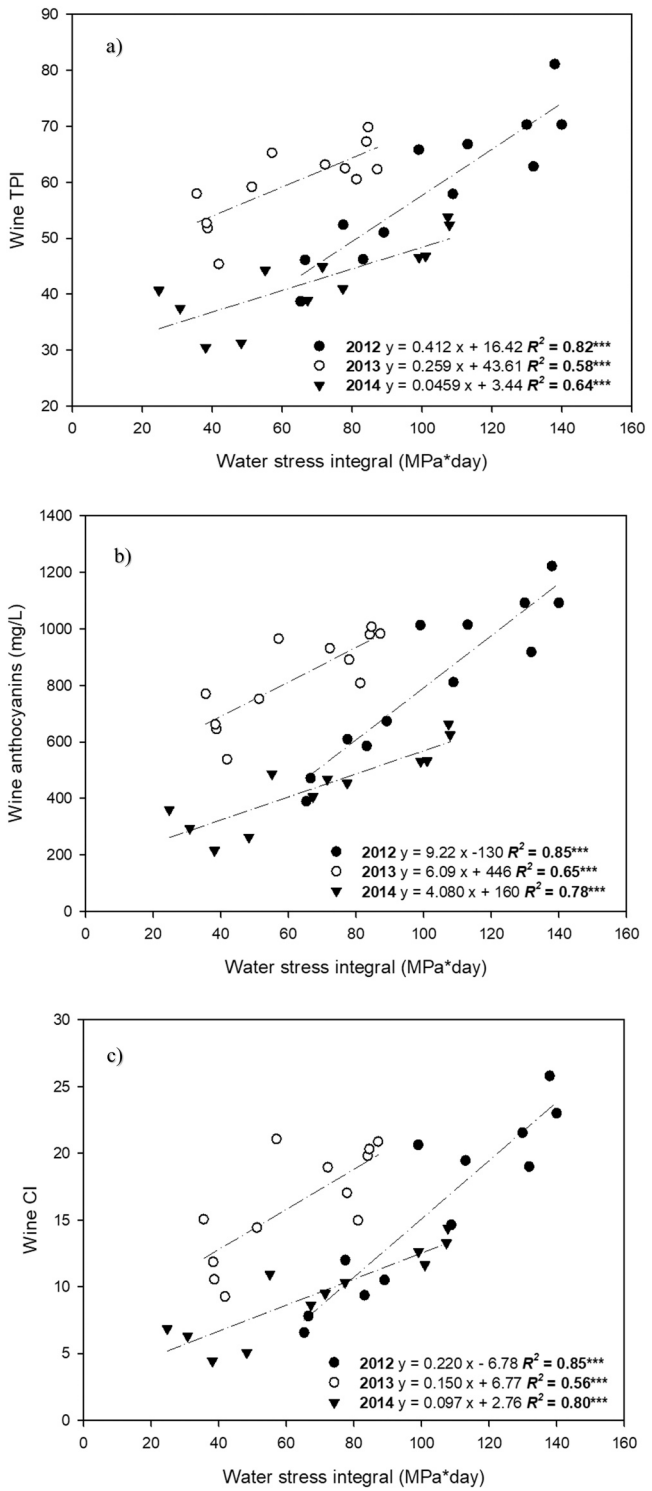


Fig. 2. Relationship of the water stress integral (MPa*day) calculated from stem water potential measured at mid-day and (a) total polyphenol index, anthocyanin content (mg L^{-1}) (b), and color intensity (c) in Bobal wines of the 2012, 2013 and 2013 vintages. Lines of linear regression and values of 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.

selectively enhancing the skin tissue absolute mass (Matthews and Kriedemann, 2006). Thus, Kyraleou et al. (2016), in a Syrah vineyard in Greece, observed that with water limitation the concentration of berry skin anthocyanins significantly increased, but these differences were maximum 2–3 weeks after veraison and decreased thereafter to reach similar levels at harvest. Matthews et al. (1990) and Nadal and Arola (1995) showed that when 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 both the concentration (amount per unit fresh weight) and the absolute content (amount per berry) of skin and seed phenolics were affected by the RDI regimes. Also, in an experiment carried out in pots with the Shiraz variety, Ojeda et al. (2002) showed that moderate water deficits increased 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 were 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 as compared to the well-watered 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 had a higher seed tannin content than the irrigation treatments, even if grape seed TPI and the weight of 100 grape seeds were higher from plants the irrigated than from the non-irrigated treatments (Table 3). Similar to our results, Casassa et al. (2015) observed that their continuous water deficit Cabernet Sauvignon grapes increased the seed tannins values. They suggested that the higher seed tannin content observed in their full deficit treatment compared to the other RDI treatments, was in partial due to the smaller grape weight. In our case, berries from the FI treatment had a lower seed weight percentage *versus* total berry weight than berries under the water restriction treatments (Table 3), even though the berry size was higher in FI (Pérez-Álvarez et al., 2021). Pastor del Rio and Kennedy (2006) observed that seed weight and the number of seeds per grape determined the seed tannin concentration. 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 grape size overrode this effect, thereby enhancing overall the level of seed tannin. However, Koundouras et al. (2009), Roby et al. (2004) and Bonada et al. (2015) observed that the water deficit did not modify the tannins of the Cabernet Sauvignon and Shiraz grape seeds, respectively, despite its effect on the weight of the berry. On their part, Kyraleou et al. (2017) and Bonada et al. (2015), suggested that the reduction of seed total tannins content observed under their non-irrigated and deficit irrigated conditions, was related to the increase in temperature observed on the berries from those treatments. Bonada et al. (2015) suggested that the heating of berries reduced tannins by 20% as compared to those under ambient conditions. Also, Kennedy et al. (2000) observed that the seed flavan-3-ols (subunit that conform the tannins) content at harvest in Cabernet Sauvignon decreased with the water limitation, while Chacón et al. (2009) reported that these compounds increased in Merlot seed with water deficiency. On the other hand, Genebra et al. (2004) suggested that although several genes from the biosynthetic pathway of flavan-3-ols were up-regulated, the levels of Tempranillo seed tannins were not affected by the irrigation treatments.

3.3. Concentration of skin and seed polymeric proanthocyanidins

The total condense tannins, analyzed with the proanthocyanidin mean degree of polymerization (mDP), the percentage of galloylation (% 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 (Palcual et al., 2016). Thus, as observed in the results (Table 4), tannins from skin are generally larger, with a higher mDP, while tannins from seed are shorter, with a lower mDP, according to the studies by Chira et al. (2009), Bordiga et al. (2011), and Pascual et al.

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 treatment (T), 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's 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.

(2016). Therefore, the mDP of skin tannin values were higher than those of seeds, and are perceived as astringency in the wine (Harrison, 2018), unlike the seed tannins, which contribute to wine bitterness (Vander-Weide 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 the FI samples than in Rainfed, and the grape skin %G was also lower with the Rainfed treatment. Therefore, it seems that the water deficit regime decreased the polymerization of tanins and perhaps even reduced wine astringency with respect to the FI treatment. According to García-Esparza et al. (2018), the wine astringency is linked, among others factors, with the berries tannin mDP. Therefore, great values of grape mDP 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 which enhance mDP, as shown in the Kyraleou et al. (2017) study, where their non-irrigated Syrah grapes had a higher astringency with a higher mDP in the polymeric skin fraction, than grapes from deficit irrigated and fully irrigated vines. However, Ojeda et al. (2002), in their study carried out in Syrah, found that the mDP increased with a water deficit treatment, and suggested that berry dehydration could possibly affect the sensorial quality of the wine by decreasing its astringency. On their part, Quijada-Morín et al. (2012) reported 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 galloylation increased 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 a low consistence throughout the experiment. Sivilotti et al. (2020) did not find remarkable difference between irrigation treatments on the structural characteristics of seed tannins (mDP and % galloylation) either. Nevertheless, Kyraleou et al. (2017) found a higher percentage of galloylation for seeds at harvest in their non-irrigated vines than in the deficit irrigated and fully-irrigated samples for both the oligomeric and polymeric tannins fractions.

Generally, (as it occurred in our work, Table 4) the higher degree of galloylation of the seed tannins makes them more astringent than skin tannins, although the bitter and the astringent perception of tannins is affected by their interactions with the soluble polysaccharides present in the grape must (Gil et al., 2012) 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 also observed higher average percentage values of gallylation in seeds than in skins. In the wine panel, skin tannins are traditionally regarded as more pleasant and softer and less bitter and astringent than seed tannins, maybe because of the lower proportion of galloylated subunits and the presence of prodelphinidins in the skin tannins (Vidal et al., 2003; Lisjak et al., 2020). According to the regression analysis shown by Kyraleou et al. (2017), a strong significant correlation exits between galloylation percentage (%G) and mDP for both skins and seeds. They observed that for skin tannins, a %G > 2.5, was associated with tannin monomers and oligomers (mDP < 4). In contrast, when mDP was higher than 8 (as in our Bobal grapes independently 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 values higher than 6%G only related with monomers, dimers, and trimers, while larger molecules (mDP > 6), had a lower percentage of galloylation (%G < 5). They argued that larger tannins from both skins and seeds were linked to a low ECG subunits percentage.

3.4. Grape aroma compounds

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

The interaction between both factors, the irrigation treatments and the season (year), was not significant in any of the determined

Table 5
Mean values (μg kg^{-1} 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 treatment (T), 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		
Vanillin 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		
Vanillin	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		

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

compounds, indicating that the influence of the water regime treatments steady during the years of study. The content of volatile compounds such as benzaldehyde, guaiacol, 4-ethylphenol, 4-vinylphenol, α-ionone, γ-decalactone, syringaldehyde, and vainillin increased with the application of irrigation when compared with the Rainfed vines (Table 5). In any case, the correlation between water application and the benzaldehyde, guaiacol and α-ionone content was significant in all three seasons (Fig. 3A-C, respectively). The reported relationships report for the effects of irrigation regime on the grape aroma compounds benzaldehyde, guaiacol and α-ionone, but they cannot be extrapolated to different soil and climatic conditions because of the empiric nature of the relations explored. However, the concentrations of benzanoic acid, 3-hydroxybenzaldehyde, and octanoic acid increased with DI or Rainfed treatments with respect to the highest dose of irrigation (FI). Also, the content of 2-phenylethanol and isobutyric acid decreased with the DI strategy with respect to the other two treatments (Table 5). Thus, according to Alem et al. (2019), water stress influences the synthesis of aroma compounds in different ways depending on the molecule family considered. In general, the abundance of enzymes involved in the production of aroma precursors was positively affected by water restriction (Deluc et al., 2009; Alem et al., 2019).

Therefore, in the case of benzene compounds, an important group in the grape varietal aroma which includes aromatic alcohols, aldehydes, and volatile phenols (Gómez García-Carpintero et al., 2014), the influence exerted by the irrigation treatments on Bobal grapes was diverse. Benzenoid derivatives tend to be synthetized later during grape development and are present in low quantities in berries (González-Barreiro et al., 2015). Thus, the grape benzaldehyde content, that could have a synergetic effect on wine aroma with fruity and floral notes (Gómez García-Carpintero et al., 2011), was higher during the three vintages in those grapevines that had unlimited irrigation (FI) (Table 5). The wettest and coldest conditions in 2013, could be the reason behind why the

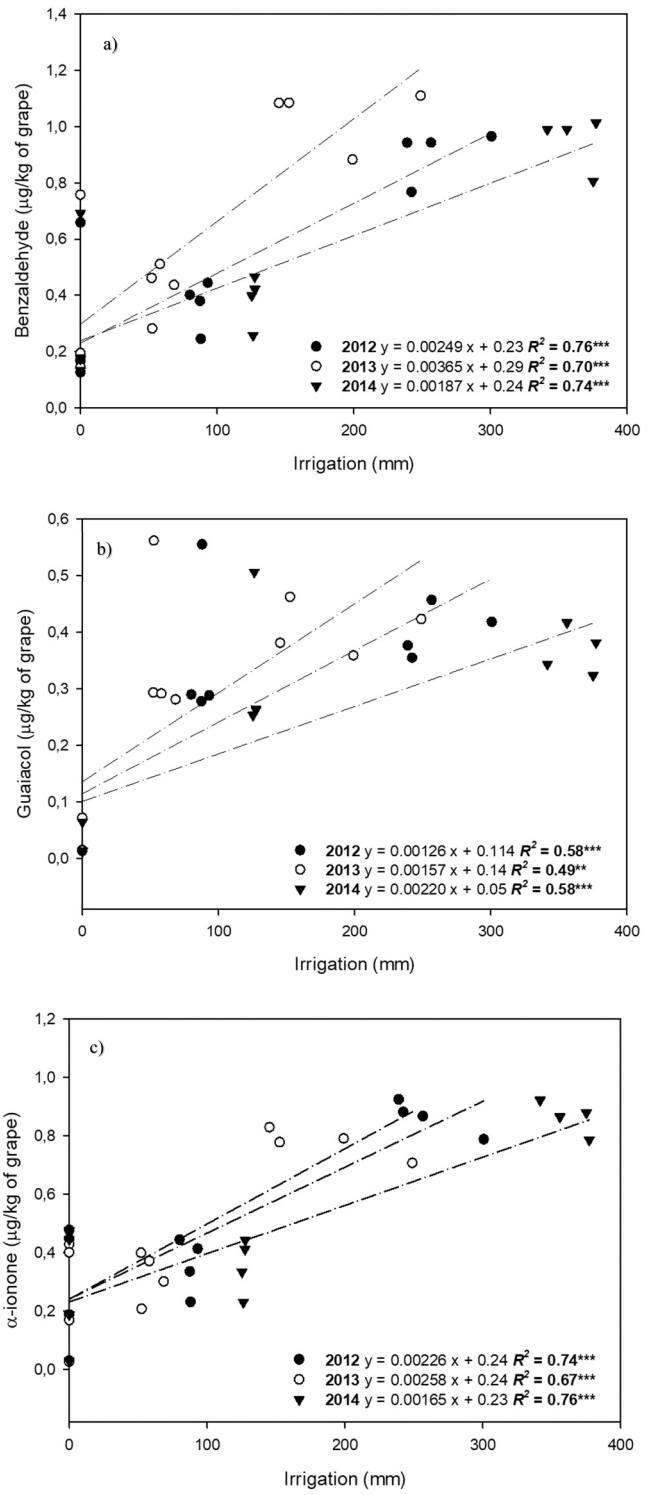


Fig. 3. Relationship of irrigation (mm) and (a) benzaldehyde, (b) guaiacol and (c) α-ionone content (µg kg^{-1} of grape) in Bobal grapes of the 2012, 2013 and 2013 vintages. Lines of linear regression, when significant, and values of the coefficient of determination (R^2) with indication of significance at $p<0.001$ (***), $p=0.05$–0.001 (**), $p < 0.05$ (*) or non significant (ns) are shown.

grapes from that year tended to had more benzaldehyde than the other seasons. This trend to vary with the availability of water makes it so that benzaldehyde, a compound which possesses a bitter-almond-like odor, characteristic of certain wines such as those produced from Gramay grapes, acts as marker of *Botrytis* infection, along with other compounds such as acetic acid, furfural and terpinen-4-ol (Fedrizzi et al., 2011). However, Ju et al. (2018) observed that the volatile compounds content after RDI treatments was linked to the amino acids concentration. They reported that the increase in benzaldehyde content in Cabernet Sauvigon grapes was closely related to the concentration of leucine, an amino acid which content increased with two deficit irrigation (70% and 80% ETc) treatments as compared to full irrigation (100% ETc) samples. On the other hand, in the present research, the 2-phenylethanol content in grapes (aromatic alcohol with a rose aroma) was reduced with the DI treatment with respect to the other irrigation regimes (Table 5), which could have an impact on the "floral" notes of grapes. While one of the precursors in grapes of this aromatic alcohol is the phenylethyl-α-D-glucopyranoside (García et al., 2003), in wines it is formed by the catabolism of the amino acid phenylalanine throughout the alcoholic fermentation process (Bell and Henschke, 2005). Contrary to our findings in which the grapes from the DI treatment had an intermediate ºBrix content at harvest with respect to grapes from the other treatments (Pérez-Álvarez et al., 2021); Fang and Qian (2012) suggested that benzyl alcohol and 2-phenylethanol synthesis was considerably enhanced during ripening. However, the content of grape benzanoic acid and 3-hydroxybenzaldehyde was higher in grapes from grapevines with restricted water availability (rainfed and DI) than those from the FI treatment (Table 5). The reduction of the level of benzanoic acid in berries from FI grapevines with respect to those from the Rainfed treatment, was of the order of 17.44%, 17.55% and 17.51% for each season, respectively.

In relation to the volatile phenols, a significant effect of irrigation was observed; guaiacol and 4-vinylphenol showed the same pattern; grapes irrigated had higher values than the Rainfed grapes; 4-ethylphenol was the highest with the DI strategy. In 2013, the 4-ethylphenol and 4-vinylphenol content was smaller than the values found in the other seasons (Table 5). Volatile phenols play a significant role in wine aroma, although depending on their concentrations, their influence on the final wine will vary (Gómez García-Carpintero et al., 2011). However, as the enzyme that catalyses their formation is inhibited by catechins and catechin tannins, which are abundant in red wines, the volatile phenols content created in red wines are generally much lower than those in white and rosé wines, although in the corresponding red musts, the contents in hydroxycinnamic precursors are higher (Chatonnet et al., 1993). Thus, alike other red wines that contain mostly very low levels of vinylphenols, in our samples the 4-vinylphenol content was higher than 4-ethylphenols, as observed by Vilanova et al. (2013) in their young white wines and Siero-Sampedro et al. (2019) in their young red wines of the Mencía variety.

Regarding α-ionone, a C-13 norisoprenoid related to tobacco flavour, an increase was observed in all the seasons on the FI grapes with respect to the other two treatments (Table 5). This could be related to the fact that carotenoids, from which the norisoprenoids are derived through their biodegradation, are mainly located in the grape skin, whose weight was higher in the FI treatment, even if the % skin weight/total berry weight ratio was lower, than in the water deficit treatments (Table 2). Also, Savoi et al. (2016) observed a greater degradation of carotenoids of white grapes under water deficit. In contrast, authors such as Deluc et al. (2009); Song et al. (2012), and Savoi et al., (2016, 2017) reported that in general, water deficit can increase the C13-norisoprenoid concentration by modulating the structural and regulatory genes involved in the biosynthesis of volatile compounds. Sasaki et al. (2016) observed that exposing grapes to light was substantially essential for the biosynthesis of cetain norisoprenoids such as linalool, β-ionone, and β-damascenone, which was not consistent with the α-ionone concentration found in our trial. In their Merlot wines, Ou et al. (2010) did not observe differences in β-ionone concentration among irrigation treatments in any of the three years studied. In a Cabertnet Sauvignon assay in which stressed plants received 66% of the water received by the control ones due to a partial root zone drying system, Bindon et al. (2007) reported that the concentration of three important C13 norisoprenoids (β-damascenone, β-ionone, and 1,1,6-trimethyl-1,2-dihydronaphthalene) was enhanced

by water stress treatments over the two seasons studied. Nevertheless, when the results were expressed in terms of ng/berry instead of concentration (ng/g), these authors did not find significant differences between treatments. Thus, they reported that perhaps the differences observed in the concentration results were due to changes in the volume and/or grape weight as a consequence of water limitation. Also, Koundouras et al. (2009) and Alem et al. (2019) suggested that the greater concentration of aroma molecules in grapes is sometimes due to a berry size limitation induced by water stress.

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

Table 5 shows the increases in vanillin and syringaldehyde content with the irrigation treatments. However, the vanillin content was highest with the DI treatment, and syringaldehyde with the FI treatment as compared to grapes from the other two treatments. Both phenolic aldehydes compounds, vanillin, and syringaldehyde, possess vanilla-like fragrances. In the family of the vanillin derivatives, there are compounds whose presence in wine in large quantities is due to their extraction from the wood during aging, being much smaller as compared to the amounts released by the hydrolysis of their glycosidic precursors. However, in non-aged wines, the reserve of aromatic potential of the precursors can have a subtle influence on the wines aroma and flavor.

The content of the fatty acids determined in the grape samples (isobutyric and octanoic acids) varied in different ways with the irrigation treatment; isobutyric acid increased with the Rainfed treatment and also with the maximum irrigation dose, however, the isobutyric octanoic acid increased in the treatments where less water content was applied (Table 5). Gómez García-Carpintero et al. (2011) also found isobutyric acid and octanoic acid among the most abundant acids in their Bobal wines. Fatty acids are found in berries, esterified in the form of phospholipids, neutral lipids, and glycolipids (Serrano de la Hoz, 2014). It is in the grape skins where most of the fatty acids are found, with their content being between 1.5 and 3 times higher than in the pulp (Bayonove, 2003). Fatty acids have been described with fruity, cheesy, fatty, and rancid notes (Rocha et al., 2004). Deluc et al. (2009) reported that water deficit affected, among others, the fatty acid metabolic pathways and, although they did not provide aroma precursors, their analysis showed that water deficiency had an effect on the amount of enzymes involved in the aroma precursors production (Alem et al., 2019).

On the other hand, Hernández-Orte et al. (2015) showed that the vintage introduced differences in most of the compounds analyzed in their study, with most of the precursors synthesized in warmer years and under more sun-exposed grapes in Tempranillo, Merlot, and Gewurztraminer varieties. As aforementioned, in our case, the vintage only affected some of the aromatic compounds (volatile phenols) detected in Bobal grapes (Table 5). However, in their white grape varieties studied, Bouzas-Cid et al. (2018a, 2018b) and Vilanova et al. (2019b), reported that the volatile organic compounds were more influenced by the inter-annual variation than by the in-season variation due to irrigation treatments. Bouzas-Cid et al. (2018a) also reported that mild or moderate levels of water deficit had little or no effects on the composition of the must and wine or its sensory properties.

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

4. Conclusions

This study shows the important role of irrigation regimes on grapevine cv. Bobal wine composition and grape quality parameters. For grapes harvested at a similar time, wines from rainfed or deficit irrigated vines were more concentrated in terms of alcohol and phenolic composition, resulting in a much higher colour content. This was not only due to a dilution effect due to the positive effects of irrigation on grape weight, but also because of a higher content of phenolic compounds in seeds and particularly skin tissues. The percentage of the skin and seed weight compared to the total weight of the grapes, as well as the skin anthocyanins content and seed and skin tannins, were higher in the less-irrigated treatments. The degree of polymerization (mDP, aMW) of the skin tannins, and the percentage of galloylation (%G), were lower in the Rainfed or even in DI grapes with respect to FI ones. This leads us to think that the astringency perception and possibly the bitterness sensation (since the seed bitterness can be compensated by the milder bitterness of the skin tannins) of wines from Bobal grapes under Rainfed and DI regime will be lower than those of the FI grapevines.

In addition, it was also demonstrated that the irrigation regime affects the grape aroma precursors and therefore determines the final wine sensory attributes. However, water deficit affects the biosynthesis of aroma compounds in different ways, depending on the molecule family considered. Thus, the grapes from the deficit irrigation strategy were richer than those from the Rainfed treatment, in volatile phenols content, which plays a significant role in wine aroma, although their effect on the final wine will be different depending on their concentrations. Also, the grapes from the deficit irrigation regime had higher benzanoic acid, 3-hydroxybenzaldehyde, and octanoic acid concentrations than grapes from the unlimited water supply grapevines, which could be attributed to a change caused by the water deficit on the metabolic pathways of these groups of compounds. Since the aroma precursors will mark the sensory attributes of the wine, it has been observed that perhaps the correct management of water supply for 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, thereby allowing for an increase in yield as compared to rainfed vines, as reported in our companion study by Pérez-Álvarez et al. (2021).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

E.P. P-Á. thanks the Spanish Government for her postdoctoral contract. This work was supported by the Spanish Ministry of Economy and Competitiveness with FEDER co-financing [grant numbers AGL-2014-54201-C4-4-R and AGL2017-83738-C3-3-R], CajaMar and Fundación Lucio Gil de Fagoaga.

References

Alem, H., Rigou, P., Schneider, R., Ojeda, H., Torregrosa, L., 2019. Impact of agronomic practices on grape aroma composition: a review. J. Sci. Food Agr. 99 (3), 975–985. https://doi.org/10.1002/jsfa.9327.

Bayonove, C., 2003. El aroma varietal: El potencial aromático de la uva. In: Fancy, C. (Ed.), Enología: Fundamentos científicos y tecnológicos. Ed Mundi-Prensa, Madrid, pp. 137–146.

Bell, S.J., Henschke, P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11, 242–295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x.

Bindon, K.A., Dry, P.R., Loveys, B.R., 2007. Influence of plant water status on the production of C13-norisoprenoid precursors in *Vitis vinifera* L. cv. Cabernet sauvignon grape berries. J. Agric. Food Chem. 55, 4493–4500. https://doi.org/10.1021/jf063331p.

Bindon, K.A., Kennedy, J.A., 2011. Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. J. Agric. Food Chem. 59, 2696–2707. https://doi.org/10.1021/jf1047207.

Blouin, J., 1992. Tecniques d analyses des moûtes et des vins. Ed. Dujardin Salleron, pp.199–201.

Bonada, M., Jeffery, D.W., Petrie, P.R., Moran, M.A., Sadras, V.O., 2015. Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. Aust. J. Grape Wine Res. 21, 240–253. https://doi.org/10.1111/ajgw.12142.

Bordiga, M., Travaglia, F., Locatelli, M., Coisson, J.D., Arlorio, M., 2011. Characterization of polymeric skin and seed proanthocyanidins during ripening in six *Vitis vinifera* L. cv. Food Chem. 127, 180–187. https://doi.org/10.1016/j.foodchem.2010.12.141.

Bouzas-Cid, Y., Falque, E., Orriols, I., Miras-Avalos, J.M., 2018a. Effects of irrigation over three years on the amino acid composition of Treixadura (*Vitis vinifera* L.) musts and wines, and on the aromatic composition and sensory profiles of its wines. Food Chem. 240, 707–716. https://doi.org/10.1016/j.foodchem.2017.08.013.

Bouzas-Cid, Y., Trigo-Cordoba, E., Falque, E., Orriols, I., Miras-Avalos, J.M., 2018b. Influence of supplementary irrigation on the amino acid and volatile composition of Godello wines from the Ribeiro Designation of Origin. Food Res. Int. 111, 715–723. https://doi.org/10.1016/j.foodres.2018.05.074.

Bucchetti, B., Matthews, M.A., Falginella, L., Peterlunger, E., Castellarin, S.D., 2011. Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. Sci. Hortic. 128, 297–305. https://doi.org/10.1016/j.scienta.2011.02.003.

Caldwell, M.M., Ballaré, C.L., Bornman, J.F., Flint, S.D., Björn, L.O., Teramura, A.H., Kulandaivelu, G., Tevini, M., 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climate change factors. Photochem. Photobiol. Sci. 2, 29–38. https://doi.org/10.1039/B211159B.

Cancela, J.J., Trigo-Córdoba, E., Martínez, E.M., Reya, B.J., Bouzas-Cid, Y., Fandiño, M., Mirás-Avalos, J.M., 2016. Effects of climate variability on irrigation scheduling in white varieties of *Vitis vinifera* L. of NW Spain. Agric. Water Manag. 170, 99–109. https://doi.org/10.1016/j.agwat.2016.01.004.

Casassa, L.F., Keller, M., Harbertson, J.F., 2015. Regulated deficit irrigation alters anthocyanins, tannins and sensory properties of Cabernet Sauvignon grapes and wines. Molecules 20, 7820–7844. https://doi.org/10.3390/molecules20057820.

Castellarin, S.D., Di Gaspero, G., 2007. Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. BMC Plant Biol. 7, 46. https://doi.org/10.1186/1471-2229-7-46.

Castellarin, S.D., Matthews, M.A., di Gaspero, G., Gambetta, G.A., 2007b. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta 227, 101–112. https://doi.org/10.1007/s00425-007-0598-8.

Chacón, J.L., Garcia, E., Martinez, J., Romero, R., Gomez, S., 2009. Impact of the vine water status on the berry and seed phenolic composition of 'Merlot' (*Vitis vinifera* L.) cultivated in a warm climate: consequence for the style of wine. Vitis 48, 7–9. https://doi.org/10.5073/vitis.2009.48.7-9.

Chassy, A.W., Adams, D.O., Laurie, V.F., Waterhouse, A.L., 2012. Tracing phenolic biosynthesis in *Vitis vinifera* via *in situ* C-13 labeling and liquid chromatography–diode-array detector–mass spectrometer/mass spectrometer detection. Anal. Chim. Acta 747, 51–57. https://doi.org/10.1016/j.aca.2012.08.012.

Chatonnet, P., Dubourdieu, D., Boidron, J.N., Lavigne, V., 1993. Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. J. Sci. Food Agric. 62, 191–202. https://doi.org/10.1002/jsfa.2740620213.

Chira, K., Schmauch, G., Saucier, C., Fabre, S., Teissedre, P.L., 2009. Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). J. Agric. Food Chem. 57, 545–553. https://doi.org/10.1021/jf802301g.

Chira, K., Pacella, N., Jourdes, M., Teissedre, P.L., 2011. Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. Food Chem. 126, 1971–1977. https://doi.org/10.1016/j.foodchem.2010.12.056.

Cohen, S.D., Kennedy, J.A., 2010. Plant metabolism and the environment: implications for managing phenolics. Crit. Rev. Food Sci. Nutr. 50, 620–643. https://doi.org/10.1080/10408390802603441.

Curko, N., Kovacevic Ganic, K., Gracin, L., Dapic, M., Jourde, M., Teissedre, P.L., 2014. Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. Food Chem. 145, 15–22. https://doi.org/10.1016/j.foodchem.2013.07.131.

Deloire, A., Kraeva, E., Dai, G.H., Renault, A.S., Rochard, J., Chatelain, C., Carbonneau, A., Andary, C., 1998. Les mécanismes de défense de la vigne. Des utilisations possibles pour lutter contre les pathogènes. Phytoma 510, 46–51. ISSN: 0048-4091.

Deloire, A., Ojeda, H., Kraeva, E., Carbonneau, A., Andary, C., 1999. L'Autodefense des baies de raisin: Une nouvelle composante de la qualité? Rev. Oenol. Tech. Vit. Oenol. 91, 5–7.

Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet, J., Wheatley, M.D., Schlauch, K.A., Merillon, J.M., Cushman, J.C., Cramer, G.R., 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genom. 10, 212. http://www.biomedcentral.com/1471-2164/10/212.

D'Onofrio, C., Matarese, F., Cuzzola, A., 2018. Effect of methyl jasmonate on the aroma of Sangiovese grapes and wines. Food Chem. 242, 352–361. https://doi.org/10.1016/j.foodchem.2017.09.084.

Esteban, M.A., Villanueva, M.J., Lisarrague, J.R., 2001. Effect of irigation on changes in the anthocyanin composition of the skin of cv. Tempranillo (*Vitis vinifera* L.) grape berries during ripening. J. Sci. Food Agr. 81, 409–420. https://doi.org/10.1002/1097-0010(200103)81:4<409::AID-JSFA830>3.0.CO;2-H.

Fang, Y., Qian, M.C., 2012. Development of C6 and other volatile compounds in Pinot noir grapes determined by stir bar sorptive extraction-GC-MS. ACS Symp. Ser. 1104, 81–99. https://doi.org/10.1021/bk-2012-1104.ch006.

Fedrizzi, B., Tosi, E., Simonato, B., Finato, F., Cipriani, M., Caramia, G., Zapparoli, G., 2011. Changes in wine aroma composition according to botrytized berry percentage: a preliminary study on Amarone wine. Food Technol. Biotechnol. 49 (4), 529–535.

Feng, H., Skinkis, P.A., Qian, M.C., 2017. Pinot noir wine volatile and anthocyanin composition under different levels of vine fruit zone leaf removal. Food Chem. 214, 736–744. https://doi.org/10.1016/j.foodchem.2016.07.110.

García, E., Chacón, J., Martínez, J., Izquierdo, P., 2003. Changes in volatile compounds during ripening in grapes of Airén, Macabeo and Chardonnay white varieties grown in La Mancha region (Spain). Food Sci. Technol. Internsh. 9 (1), 33–41. https://doi.org/10.1177/1082013203009001006.

García-Esparza, M.J., Abrisqueta, I., Escriche, I., Intrigliolo, D.I., Álvarez, I., Lizama, V., 2018. Volatile compounds and phenolic composition of skins and seeds of 'Cabernet Sauvignon' grapes under different deficit irrigation regimes. Vitis 57, 83–91. https://doi.org/10.5073/vitis.2018.57.83-91.

Genebra, T., Santos, R.R., Francisco, R., Pinto-Marijuan, M., Brossa, R., Serra, A.T., Duarte, C.M.M., Chaves, M.M., Zarrouk, O., 2014. Proanthocyanidin accumulation and biosynthesis are modulated by the irrigation regime in Tempranillo seeds. Int. J. Mol. Sci. 15, 11862–11877. https://doi.org/10.3390/ijms150711862.

Gil, M., Kontoudakis, N., González, E., Esteruelas, M., Fort, F., Canals, J.M., Zamora, F., 2012. Influence of grape maturity and maceration length on color, polyphenolic composition, and polysaccharide content of Cabernet Sauvignon and Tempranillo wines. J. Agric. Food Chem. 60, 7988–8001. https://doi.org/10.1021/jf302064n.

Glories, Y., 1978. Recherches sur la matière colorante des vins rouges. Thèse à L'Université de Bordeaux II, 1: 187–195.

Gómez García-Carpintero, E., Sánchez-Palomo, E., González Viñas, M.A., 2014. Volatile composition of Bobal red wines subjected to alcoholic/malolactic fermentation with oak chips. LWT - Food Sci. Technol. 55, 586–594. https://doi.org/10.1016/j.lwt.2013.10.024.

Gómez García-Carpintero, E., Sánchez-Palomo, E., González-Viñas, M.A., 2011. Aroma characterization of red wines from cv. Bobal grape variety grown in LaMancha region. Food Res. Int. 44, 61–71. https://doi.org/10.1016/j.foodres.2010.11.013.

González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., Simal-Gándara, J., 2015. Wine aroma compounds in grapes: a critical review. Crit. Rev. Food Sci. Nutr. 55, 202–218. https://doi.org/10.1080/10408398.2011.650336.

Harrison, R., 2018. Practical interventions that influence the sensory attributes of red wines related to the phenolic composition of grapes: a review. Int. J. Food Sci. Technol. 53, 3–18. https://doi.org/10.1111/ijfs.13480.

Hernandez-Orte, P., Concejero, B., Astrain, J., Lacau, B., Cacho, J., Ferreir, V., 2015. Influence of viticulture practices on grape aroma precursors and their relation with wine aroma. J. Sci. Food Agric. 2015 (95), 688–701. https://doi.org/10.1002/jsfa.6748.

Hochberg, U., Degu, A., Cramer, G.R., Rachmilevitch, S., Fait, A., 2015. Cultivar specific metabolic changes in grapevines berry skins in relation to deficit irrigation and hydraulic behavior. Plant Physiol. Biochem. 88, 42–52. https://doi.org/10.1016/j.plaphy.2015.01.006.

Holt, H.E., Birchmore, W., Herderich, M.J., Iland, P.G., 2010. Berry phenolics in Cabernet Sauvignon (*Vitis vinifera* L.) during late-stage ripening. Am. J. Enol. Vitic. 61 (3), 285–299. ISSN: 00029254.

Intrigliolo, D.S., Castel, J.R., 2009. Response of *Vitis vinifera* cv. 'Tempranillo' to partial rootzone drying in the field: water relations, growth, yield and fruit and wine quality. Agric. Water Manag. 96, 282–292. https://doi.org/10.1016/j.agwat.2008.08.001.

Intrigliolo, D.S., Castel, J.R., 2010. Response of grapevine cv. 'Tempranillo' to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. Irrig. Sci. 2010 (28), 113–125. https://doi.org/10.1007/s00271-009-0164-1.

Intrigliolo, D.S., Pérez, D., Risco, D., Yeves, A., Castel, J.R., 2012. Yield components and grape composition responses to seasonal waterdeficits in Tempranillo grapevines. Irrig. Sci. 30, 339–349. https://doi.org/10.1007/s00271-012-0354-0.

Intrigliolo, D.S., Lizama, V., Garcìa-Esparza, M.J., Abrisqueta, I., Alvarez, I., 2016. Effects of post-veraison irrigation regime on Cabernet Sauvignon grapevines in Valencia, Spain: yield and grape composition. Agr. Water Manag. 170, 110–119. https://doi.org/10.1016/j.agwat.2015.10.020.

Jia, H., Wang, S., Lin, H., Satio, T., Ampa, K., Todoroki, Y., Kondo, S., 2018. Effects of abscisic acid agonist or antagonist applications on aroma volatiles and anthocyanin biosynthesis in grape berries. J. Hortic. Sci. Biotechnol. 98, 1140–1152. https://doi.org/10.1080/14620316.2017.1379364.

Ju, Y.-L., Yue, X.-F., Zhao, X.-F., Zhao, H., Fang, Y.-L., 2018. Physiological, micro-morphological and metabolomic analysis of grapevine (*Vitis vinifera* L.) leaf of plants under water stress. Plant Physiol. Biochem. 130, 501–510. https://doi.org/10.1016/j.plaphy.2018.07.036.

Junquera, P., Lissarrague, J.R., Jiménez, L., Linares, R., Baeza, P., 2012. Long-term effects of different irrigation strategies on yield components, vine vigour, and grape composition in cv. Cabernet-Sauvignon (*Vitis vinifera* L.). Irrig. Sci. 30, 351–361. https://doi.org/10.1007/s00271-012-0348-y.

Kallithraka, S., Tsoutsouras, E., Tzourou, E., Lanaridis, P., 2006. Principal phenolic compounds in Greek red wines. Food Chem. 99 (4), 784–793. https://doi.org/10.1016/j.foodchem.2005.07.059.

Keller, M., Smithyman, R.P., Mills, J.M., 2008. Interactive effects of deficit irrigation and crop load on cabernet sauvignon in an arid climate. Am. J. Enol. Vitic. 59 (3), 221–234.

Kennedy, J.A., Matthews, M.A., Waterhouse, A.L., 2000. Changes in grape seed polyphenols during fruit ripening. Phytochemistry 55, 77–85. https://doi.org/10.1016/S0031-9422(00)00196-5.

Kennedy, J.A., Jones, G.P., 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. J. Agric. Food Chem. 49, 1740–1746. https://doi.org/10.1021/jf001030o.

Kennedy, J.A., Matthews, M.A., Waterhouse, A., 2002. Effect of maturity and vine water status on grape skin and wine flavonoids. Am. J. Enol. Vitic. 53, 269–274. ISSN: 00029254.

Kyraleou, M., Kallithraka, S., Koundouras, S., Chira, K., Haroutounian, S., Spinthiropoulou, H., Kotseridis, Y., 2015. Effect of vine training system on the phenolic composition of red grapes (*Vitis vinifera* L. cv. Xinomavro). J. Int. Sci. Vigne Vin 49, 71–84. https://doi.org/10.20870/oeno-one.2015.49.2.92.

Kyraleou, M., Koundouras, S., Kallithraka, S., Theodorou, N., Proxenia, N., Kotseridis, Y., 2016. Effect of irrigation regime on anthocyanin content and antioxidant activity of *Vitis vinifera* L. cv. Syrah grapes under semiarid conditions. J. Sci. Food Agric. 96, 988–996. https://doi.org/10.1016/j.foodchem.2016.02.052.

Kyraleou, M., Kallithraka, S., Theodorou, N., Teissedre, P.L., Kotseridis, Y., Koundouras, S., 2017. Changes in tannin composition of Syrah grape skins and seeds during fruit ripening under contrasting water conditions. Molecules 22 (1453), 1–18. https://doi.org/10.3390/molecules22091453.

Koundouras, S., Marinos, V., Gkoulioti, A., Kotseridis, Y., Van Leeuwen, C., 2006. Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. J. Agric. Food Chem. 54, 5077–5086. https://doi.org/10.1021/jf0605446.

Koundouras, S., Zidimitriou, E., Karamolegkou, M., Eimopoulou, E., Kallithraka, S., Tsialtas, J.T., Zioziou, E., Nikolaou, N., Kotseridis, Y., 2009. Irrigation and rootstock effects on the phenolic concentration and aroma potential of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. J. Agric. Food Chem. 57, 7805–7813. https://doi.org/10.1021/jf901063a.

Lisjak, K., Lelova, Z., Žigon, U., Bolta, S.V., Teissedred, P.L., Vanzo, A., 2020. Effect of extraction time on content, composition and sensory perception of proanthocyanidins in wine-like medium and during industrial fermentation of Cabernet Sauvignon. J. Sci. Food Agric. 100, 1887–1896. https://doi.org/10.1002/jsfa.10189.

Loscos, N., Hernandez-Orte, P., Cacho, J., Ferreira, V., 2007. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. J. Agric. Food Chem. 55, 6674–6684. https://doi.org/10.1021/jf0702343.

Matthews, M.A., Ishii, R., Anderson, M.M., O'Mahony, M., 1990. Dependence of wine sensory attributes on vine water status. J. Sci. Food Agric. 51, 321–335. https://doi.org/10.1002/jsfa.2740510305.

Matthews, M.A., Kriedemann, P.E., 2006. Water deficit, yield, and berry size as factors for composition and sensory attributes of red wine. In Proceedings of the Australian Society of Viticulture and Oenology 'Finishing the Job'—Optimal Ripening of Cabernet Sauvignon and Shiraz, Adelaide, Australia, 21 July 2006; pp. 46–54.

Mercurio, M.D., Smith, P.A., 2008. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. J. Agric. Food Chem. 56 (14), 5528–5537. https://doi.org/10.1021/jf8008266.

Mirás-Avalos, J.M., Buesa, I., Yeves, A., Pérez, D., Risco, D., Castel, J.R., Intrigliolo, D.S., 2019. Unravelling the effects of berry size on 'tempranillo' grapes under different field practices. Ciência Técnica Vitiv 34, 1–15. https://doi.org/10.1051/CTV/20193401001.

Nadal, M., Arola, J., 1995. Effect of limited irrigation on the composition of must and wine of cabernet sauvignon under semi-arid conditions. Vitis 34, 151–154. ISSN: 00427500.

OIV, 2003. Compendium of Internationals Methods of Wine and Must Analysis. Organisation Internationale de la Vigne et du Vin (OIV), Paris, France.

Ojeda, H., Deloire, A., Carbonneau, A., 2001. Influence of water deficits on grape berry growth. Vitis 40, 141–145. ISSN: 00427500.

Ojeda, H., Andary, C., Creaba, E., Carbonneau, A., Deloire, A., 2002. Influence of pre-and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. Am. J. Enol. Vitic. 53, 261–267. ISSN: 00029254.

Ollé, D., Guiraud, J.L., Souquet, J.M., Terrier, N., Ageorges, A., Cheynier, V., Verries, C., 2011. Effect of pre- and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. Aust. J. Grape Wine Res. 17, 90–100. https://doi.org/10.1111/j.1755-0238.2010.00121.x.

Ou, C., Xiaofen, D., Shellie, K., Ross, C., Qian, M.C., 2010. Volatile compounds and sensory attributes of wine from cv. Merlot (*Vitis vinifera* L.) grown under differential levels of water deficit with or without a kaolin-based, foliar reflectant particle Film. J. Agric. Food Chem. 2010 (58), 12890–12898. https://doi.org/10.1021/jf102587x.

Pascual, O., González-Royo, E., Gil, M., Gómez-Alonso, S., García-Romero, E., Canals, J. M., Hermosín-Gutíerrez, I., Zamora, F., 2016. Influence of grape seeds and stems on wine composition and astringency. J. Agr. Food Chem. 64, 6555–6566. https://doi.org/10.1021/acs.jafc.6b01806.

Pastor del Rio, J.L., Kennedy, J.A., 2006. Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot Noir grapes and extraction into wine. Am. J. Enol. Vitic. 57, 125–132.

Pérez-Álvarez, E.P., 2017. Cover crops in viticulture: astrategy to modify grape and wine phenolic composition. Chapter 7. Phenolic Compounds: Types, Effects and Research. Nova Science Publishers, Inc, pp. 179–212.

Pérez-Álvarez, E.P., Intrigliolo Molina, D.S., Vivaldi, G.A., García-Esparza, M.J., Lizama, V., Álvarez, I., 2021. Effects of the irrigation regimes on grapevine cv. Bobal in a Mediterranean climate: I. Water relations, vine performance and grape composition. Agric. Water Manag. 248, 106772 https://doi.org/10.1016/j.agwat.2021.106772.

Petrie, P.R., Cooley, M.N., Clingeleffer, P.R., 2004. Effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera*L.) in the current and following season. Aust. J. Grape Wine Res. 10, 203–215. https://doi.org/10.1111/j.1755-0238.2004.tb00024.x.

Pinasseau, L., Vallverdú-Queralt, A., Verbaere, A., Roques, M., Meudec, E., Le Cunff, L., Péros, J.-P., Ageorges, A., Sommerer, N., Boulet, J.-C., Terrier, N., Cheynier, V., 2017. Cultivar diversity of grape skin polyphenol composition and changes in response to drought investigated by lc-ms based metabolomics. Front. Plant Sci. 8, 1826. https://doi.org/10.3389/fpls.2017.01826.

Pons, A., Allamy, L., Schüttler, A., Rauhut, D., Thibon, C., Darriet, P., 2017. What is the expected impact of climate change on wine aroma compounds and their precursors in grape? Oeno One 51 (2), 141–146. https://doi.org/10.20870/oeno-one.2016.0.0.1868.

Prieur, C., Rigaud, J., Cheynier, V., Moutounet, M., 1994. Oligomeric and polymeric procyanidins from grape seeds. Phytochemistry 36 (3), 781–784. https://doi.org/10.1016/S0031-9422(00)89817-9.

Quijada-Morín, N., Regueiro, J., Simal-Gándara, J., Tomás, E., Rivas-Gonzalo, J.C., Escribano-Bailón, M.T., 2012. Relationship between the sensorydetermined astringency and the flavanolic composition of red wines. J. Agric. Food Chem. 60, 12355–12361. https://doi.org/10.1021/jf3044346.

Resco, P., Iglesias, A., Bardají, I., Sotés, V., 2016. Exploring adaptation choices for grapevine regions in Spain. Reg. Environ. Change 16 (4), 979–993. https://doi.org/10.1007/s10113-015-0811-4.

Ribéreau-Gayon, P., Stonestreet, E., 1965. Determination of anthocyanins in red wine | [Le dosage des anthocyanes dans le vin rouge. Bull. Soc. Chim. Fr. 9, 9–2652. PubMed ID: 5848688.

Ribéreau-Gayon, P., Stonestreet, E., 1966. Dosage des tanins du vin rouge et détermination de leur structur. Chim. Anal. 48 (1), 188–196.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2000. Phenolic compound. Handbook of Enology. The Chemistry of Wine Stabilization and Treatments, Vol 2. Wiley, West Sessex, pp. 129–187.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2006. Handbook of enology. The Chemistry of Wine Stabilization and Treatments, Vol. 2. John Wiley & Sons, Ltd, Chichester, pp. 129–186.

Rinaldi, A., Jourdes, M., Teissedre, P.L., Moio, L., 2014. A preliminary characterization of Aglianico (*Vitis vinifera* L. cv.) grape proanthocyanidins and evaluation of their reactivity towards salivary proteins. Food Chem. 164, 142–149. https://doi.org/10.1016/j.foodchem.2014.05.050.

Roby, G., Harbertson, J.F., Adams, D.A., Matthews, M.A., 2004. Berry size and vine water deficits as factors in winegrape composi-tion: anthocyanins and tannins. Aust. J. Grape Wine Res. 10, 100–107. https://doi.org/10.1111/j.1755-0238.2004.tb00012.x.

Rocha, S.M., Rodrigues, F., Coutinho, P., Delgadillo, I., Coimbra, M.A., 2004. Volatile composition of Baga red wine assessment of the identification of the would-be impact odourants. Anal. Chim. Acta 513 (1), 257–262. https://doi.org/10.1016/j.aca.2003.10.009.

Romero, P., Fernández-Fernández, J.I., Martines-Cutillas, A., 2010. Physiological thresholds for efficient regulated deficit-irrigation management in winegrapes grown under semiarid conditions. Am. J. Enol. Vitic. 61, 300–312.

Romero, P., Gil-Muñoz, R., del Amor, F.M., Valdés, E., Fernández, J.I., Martinez-Cutillas, A., 2013. Regulated deficit irrigation based upon optimum water status improves phenolic composition in Monastrell grapes and wines. Agr. Water Manag. 121, 85–101. https://doi.org/10.1016/j.agwat.2013.01.007.

Salinas, M.R., 2013. Potencial aromático de la uva. Jornadas interregionales de innovación e investigación vitivinícola., Cuenca. Cooperativas Agroalimentarias de Castilla-La Mancha, Ed.

Salón, J.L., Chirivella, C., Castel, J.R., 2005. Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. Am. J. Enol. Vitic. 56, 1–8.

Santesteban, L.G., Miranda, C., Royo, J.B., 2011. Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv. 'Tempranillo'. Agric. Water Manag. 98, 1171–1179. https://doi.org/10.1016/j.agwat.2011.02.011.

Sasaki, K., Takase, H., Matsuyama, S., Kobayashi, H., Matsuo, H., Ikoma, G., Takata, R., 2016. Effect of light exposure on linalool biosynthesis and accumulation in grape

berries. Biosci. Biotechnol. Biochem. 80 (12), 2376–2382. https://doi.org/10.1080/09168451.2016.1217148.

Savoi, S., Wong, D.C.J., Arapitsas, P., Miculan, M., Bucchetti, B., Peterlunger, E., Fait, A., Mattivi, F., Castellarin, S.D., 2016. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.). BMC Plant Biol. 16, 67. https://doi.org/10.1186/s12870-016-0760-1.

Savoi, S., Wong, D.C.J., Degu, A., Herrera, J.C., Bucchetti, B., Peterlunger, E., Fait, A., Mattivi, F., Castellarin, S.D., 2017. Multi-omics and integrated network analyses reveal new insights into the systems relationships between metabolites, structural genes, and transcriptional regulators in developing grape berries (*Vitis vinifera* L.) exposed to water deficit. Front. Plant Sci. 8, 1124. https://doi.org/10.3389/fpls.2017.01124.

Serrano de la Hoz, K., 2014. Determinación de precursores aromáticos glicosídicos de uvas, mostos y vinos, blancos y tintos. Aplicación en bodega. Universidad de Castilla-La Mancha, Spain, p. 293 (Doctoral Thesis).

Sieiro-Sampedro, T., Figueiredo-Gonzalez, M., González-Barreiro, C., Simal-Gandara, J., Cancho-Grande, B., Rial-Otero, R., 2019. Impact of mepanipyrim and tetraconazole in Mencía wines on the biosynthesis of volatile compounds during the winemaking process. Food Chem. 300, 125223 https://doi.org/10.1016/j.foodchem.2019.125223.

Sivilotti, P., Falchi, R., Vanderweide, J., Sabbatini, P., Bubola, M., Vanzo, A., Lisjak, K., Peterlunger, E., Herrera, J.C., 2020. Yield reduction through cluster or selective berry thinning similarly modulates anthocyanins and proanthocyanidins composition in Refosco dal peduncolo rosso (*Vitis vinifera* L.) grapes. Sci. Hortic. 264, 109166 https://doi.org/10.1016/j.scienta.2019.109166.

Somers, T.C., 1971. The polymeric nature of wine pigments. Phytochemistry 10 (9), 2175–2186. https://doi.org/10.1016/S0031-9422(00)97215-7.

Song, J., Shellie, K.C., Wang, H., Qian, M.C., 2012. Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*Vitis vinifera* L.). Food Chem. 134, 841–850. https://doi.org/10.1016/j.foodchem.2012.02.193.

Souquet, J.M., Cheynier, V., Brossaud, F., Moutounet, M., 1996. Polymeric proanthocyanidins from grape skins. Phytochemistry 43, 509–512. https://doi.org/10.1016/0031-9422(96)00301-9.

VanderWeide, J., Forte, A., Peterlunger, E., Sivilotti, P., Medina-Meza, I.G., Falchi, R., Rustioni, L., Sabbatini, P., 2020. Increase in seed tannin extractability and oxidation using a freeze-thaw treatment in cool-climate grown red (*Vitis vinifera* L.) cultivars. Food Chem. 308, 125571 https://doi.org/10.1016/j.foodchem.2019.125571.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., Waters, E.J., 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. J. Sci. Food Agric. 83, 564–573. https://doi.org/10.1002/jsfa.1394.

Vilanova, M., Escudero, A., Graña, M., Cacho, J., 2013. Volatile composition and sensory properties of NorthWest Spain white wines. Food Res. Int. 54, 562–568. https://doi.org/10.1016/j.foodres.2013.07.036.

Vilanova, M., Rodríguez-Nogales, J.M., Vila-Crespo, J., Yuste, J., 2019a. Influence of water regime on yield components, must composition and wine volatile compounds of *Vitis vinifera* cv. Verde Aust. J. Grape Wine Res. 25, 83–91. https://doi.org/10.1111/ajgw.12370.

Vilanova, M., Fandino, M., Frutos-Puerto, S., Cancela, J.J., 2019b. Assessment fertigation effects on chemical composition of *Vitis vinifera* L. cv. Albarino. Food Chem. 278, 636–643. https://doi.org/10.1016/j.foodchem.2018.11.105.

Williams, L.E., Ayars, J.E., 2005. Grapevine water use and the crop coefficient are linear functions of the shaded area measured beneath the canopy. Agric. For. Meteor. 135, 201–211. https://doi.org/10.1016/j.agrformet.2005.07.010.

Woollmann, N., Hofmann, T., 2013. Compositional and sensory characterization of red wine polymers. J. Agric. Food Chem. 61, 2045–2061.

Zarrouk, O., Francisco, R., Pinto-Marijuan, M., Brossa, R., Santos, R.R., Pinheiro, C., Costa, J.M., Lopes, C., Chaves, M.M., 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (syn. Tempranillo) grapevine. Agric. Water Manag. 114, 18–29. https://doi.org/10.1016/j.agwat.2012.06.018.

Zhang, E.P., Chai, F.M., Zhang, H.H., Li, S.H., Liang, Z.C., Fan, P.G., 2017. Effects of sunlight exclusion on the profiles of monoterpene biosynthesis and accumulation in grape exocarp and mesocarp. Food Chem. 164, 242–250. https://doi.org/10.1016/j.foodchem.2017.05.127.