Is deficit irrigation with saline waters a viable alternative for winegrowers in semiarid areas?

Alejandro Martínez-Moreno[1], Eva P. Pérez-Álvarez[2], Ramón López-Urrea[3], Diego S. Intrigliolo[4], M. Reyes González-Centeno[5], Pierre-Louis Teissedre[5] and Rocío Gil-Muñoz[6]

[1] Department of Food Science and Technology, Faculty of Veterinary Sciences, University of Murcia, 30100, Murcia, Spain

[2] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Campus Universitario de Espinardo, 30100, Murcia, Spain

[3] Instituto Técnico Agronómico Provincial (ITAP), Parque Empresarial Campollano, 2ª Avda. Nº 61, 02007, Albacete, Spain

[4] Centro de Investigación sobre Desertificación (CSIC-UV-GV). Carretera CV-315, km 10.7, 46113-Moncada, Valencia, Spain

[5] Univ. Bordeaux, ISVV, EA 4577, Œnologie, 210 Chemin de Leysotte, 33140 Villenave d'Ornon, France

[6] Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), 30150, La Alberca, Murcia, Spain

ABSTRACT

Two of the main challenges of Mediterranean viticulture is low water quality and the risk of increasing concentrations of mineral salts in the root zone. This work was undertaken to study the impact of saline deficit irrigation on grape and wine phenolic composition, as well as on the sensory profile of the wines. The experiment was carried out during three consecutive years (2016-2018) in a commercial vineyard of cv. Monastrell (*Vitis vinifera* L.) grafted onto 1103P rootstock located in D.O. Jumilla (SE Spain). Three watering regimes were carried out: i) the control ("Control"): the vines were irrigated with water of standard quality, ii) Sulfate treatment ("Sul"): the vines were irrigated with saline water (Na_2SO_4 + $MgSO4$), and iii) Chloride treatment ("Chl"): the vines were irrigated with saline water ($NaCl$). The same amount of irrigation water was applied to all the treatments. The water electrical conductivity was 1.8 dS/m for Control and 5 dS/m for the saline treatments (Sul and Chl). Both the Sul and Chl treatments reduced the berry weight in all the study years compared to Control, although this difference was statistically significant in the last year only ($p \leq 0.05$). No significant differences ($p > 0.05$) were observed in the grape quality parameters. However, the saline treatments slightly increased grape total soluble solids (TSS) in two out of the three study years compared to Control. Regarding the phenolic composition, no significant differences ($p > 0.05$) among treatments were found in grapes and wines. In general, the wines from vines irrigated with saline waters received the best scores by the panel in the sensory profile analysis. The use of saline waters could be employed in the case of water scarcity, as long as the vineyard is planted on a rootstock tolerant to salinity, such as 1103P, and the vineyard soil has a texture that favours leaching.

KEYWORDS: *Vitis vinifera*, Monastrell, anthocyanins, phenolic compounds, salinity

INTRODUCTION

As a consequence of global warming, weather patterns are changing faster than anticipated, the main quantifiable changes being temperature increase and changes in precipitation regimes (IPCC, 2019). The combination of reduced rainfall and warming generates strong trends of drier conditions, particularly in Mediterranean climates. In semiarid areas with low rainfall, one of the major water-quality problems associated with irrigation is the risk of increasing concentrations of mineral salts in the root zone (Hepaksoy *et al.*, 2007). Rising soil salinisation could be a serious threat to grape growing, because most irrigated vineyards, especially those deficit-irrigated, are at risk due to the high concentration of dissolved salts in irrigation water (Keller, 2010). Therefore, the most imminent challenges that winegrowers will face, particularly in arid and semiarid environments in the Mediterranean basin, are increased drought and salinity due to higher evaporation and declining water availability (Schultz and Jones, 2010).

In the southeast of Spain, the lack of rainfall directly affects the accumulation of fresh water in aquifers and underground wells. This considerably reduces the availability of water during the summer months, coinciding with the time of highest evaporative demand and water consumption by many crops, such as grapevines. Moreover, in arid regions, groundwater is often affected by a high concentration of soluble salts due to high evapotranspiration prolonged drought periods and the dissolution of salts. In the coming years, with the progressive loss of surface water resources and increasing demographic growth and intensive agriculture, groundwater resources will gradually become more stressed, especially in arid and semiarid climates (Ranjan *et al.*, 2009).

Grape composition and wine quality are highly dependent on many factors, such as environmental, endogenous and management practices (Dai *et al.*, 2011). Of all these factors, environmental conditions play a very important role, particularly water availability. The effects of water stress on grapevine growth and berry composition have been widely studied for many combinations of rootstocks, cultivars and climate conditions (Santesteban *et al.*, 2011; van Leeuwen *et al.*, 2009). Moreover, in recent years, the effects of water deficit on secondary berry metabolites and wine composition have been assessed (Gambetta *et al.*, 2020; Savoi *et al.*, 2020). Some studies have shown that water stress significantly affects the grape composition, and therefore the wine quality (Downey *et al.*, 2003), of mainly red varieties grown in semiarid conditions (van Leeuwen *et al.*, 2009). In an interesting review article, Chaves *et al.* (2010) concluded that slight water stress produced by deficit irrigation positively influences the accumulation of secondary compounds, such as anthocyanins in berries, when compared to irrigation covering 100% of crop evapotranspiration (ET_c). Lastly, it should be noted that compositional changes in grapes induced by water stress are not always reflected in wines, and contradictory results have often been obtained in terms of the concentration of these compounds in grapes and wines (Kennedy *et al.*, 2002).

Salinity effects on yield and berry composition have mainly been studied in Australia and Israel (Walker *et al.*, 2004; Hepaksoy *et al.*, 2007), and research in other geographical areas is scarce. The use of saline water for vine irrigation may significantly affect the composition of berries and wine (Netzer *et al.*, 2014). Reported results suggested that cultivar, rootstock, salt concentration and exposure time to saline conditions are relevant factors for final berry and wine composition (Walker *et al.*, 2002; Walker *et al.*, 2004).

In general, moderate saline stress can result in earlier veraison and a higher concentration of sugar in grapes (Walker *et al.*, 2000). Furthermore, as salinity increases, the accumulation of sugar and anthocyanins decreases (Hawker and Walker, 1978). Salinity stimulates some biochemical and physiological processes in plants; for example, the production of reactive oxygen species (ROS). To reduce damage, plants have evolved complex anti-oxidative systems, involving secondary metabolites like phenolic compounds (Posmyk *et al.*, 2009). These phenolic compounds are scavengers of free radicals (Rice-Evans *et al.*, 1997) and therefore susceptible to changes under saline stress. Hirzel *et al.* (2017) observed a significant decrease in grape phenolic content in vines irrigated with saline water in California. In contrast, for Shiraz vines irrigated with saline water, Walker *et al.* (2000) found no significant effects ($p \leq 0.05$) on colour intensity, and total phenolic and total anthocyanin content.

Finally, from a sensory point of view, wines from grapes grown in saline lands or irrigated with saline waters contain high concentrations of sodium and chloride, resulting in sensory nuances of overripe aromas and undesirable flavours classified as salty, soapy and brackish (Walker *et al.*, 2010). However, Scacco *et al.* (2010) studied Nero d'Avola vines irrigated with saline waters, observing that soil salinity enhanced citric and fruity aromas.

Therefore, the lack of good quality water resources for vineyard irrigation, caused by both rain scarcity and overuse of aquifers, forces winegrowers to irrigate the vineyard with water of high saline concentration. However, little information exists about the impact of saline irrigation on wine phenolic composition in the Mediterranean area. Thus, the purpose of this study was to evaluate the effect of saline deficit irrigation on grape and wine phenolic composition (Monastrell cv.), as well as its impact on the wine sensory profile, in order to provide winegrowers of the Mediterranean area with valuable information about irrigation management decisions.

MATERIALS AND METHODS

1. Plant material and site description

The study was carried out during three consecutive seasons (2016-2018) in a commercial vineyard of cv. Monastrell (*Vitis vinifera* L.) grafted onto 1103P rootstock.

Grapevines were planted in 2007 with a spacing of 1.50 m between vines and 3.00 m between rows (2,222 vines/ha). The vineyard is located in Fuente-Álamo (Albacete, SE Spain) (38°43′ N, 1°28′ W, elevation 820 m a.s.l., and field slope of about 3 % with a NW-SE orientation). The vines were trained as a vertical trellis on a bilateral cordon system with a north-south orientation.

The soil in the vineyard was a loamy sand (56 % sand, 27 % silt and 17 % clay) with a 90 cm deep profile. The climate is typically Mediterranean; i.e., semiarid with prevalent dry and warm summers. Long-term average annual rainfall is 290 mm, with about 60 % falling during the dormant period. The total annual grass reference evapotranspiration (ET_o) is around 1,250 mm.

2. Experimental design and irrigation treatments

The experiment design consisted of three irrigation treatments (Table 1): i) the control ("Control"): grapevines were irrigated with water from a well (electrical conductivity (EC) of 1.8 dS/m); ii) Sulfate treatment ("Sul"): vines were irrigated with emulated saline water adding sulfates (Na_2SO_4 + $MgSO_4$) to the control water, and iii) Chloride treatment ("Chl"): vines were irrigated with saline water adding chlorides (NaCl) to the control water. Both saline treatments (Sul and Chl) showed the same water EC (5.0 dS/m). All treatments were applied to the same plants during the three years of the field trial. This EC was obtained by adding different concentrations of salts to "Control water". Thus, 28 meq/L of NaCl was added to the good quality water for the Chl treatment, and 11 meq/L of Na_2SO_4 and 17 meq/L of $MgSO_4$ was added for the Sul treatment. The experimental design comprised four completely randomised blocks. Each block contained all three treatments (one experimental unit per treatment and block). Each experimental unit consisted of 48 vines (4 rows x 12 vines/row). Twenty vines in the middle rows were used as the control and the surrounding perimeter vines were used as buffers (28).

Irrigation began when the grapevines stem water potential (**Ψs**) reached -0.8 MPa and it ended once the leaves had fallen (end of vegetative cycle). Irrigation scheduling was carried out weekly using the soil water balance method proposed in FAO56. Crop evapotranspiration (ET_c) was computed by multiplying the grass reference evapotranspiration (ET_o) by a crop coefficient (K_c) as reported by López-Urrea *et al*. (2012), and adjusted to water availability. The daily ET_o was calculated with the FAO56 Penman-Monteith equation using the climatic data provided by a nearby weather station (Ontur meteorological station, 38º 37' 22.3'' N, 1º 29' 44.8'' W, AB06). A drip irrigation system was used, comprising one emitter with a nominal flow rate of 3.8 L/h per linear metre of pipe.

3. Berry size and must composition

Time of harvest was defined as being when the grapes reached optimum maturity, as laid down in the rules of the Appellation of Origin (A.O.) Jumilla (ºBrix/acidity ratio). Berry mass was determined on random samples of 100 berries per replicate (400 berries/treatment). After harvest, grapes were crushed and must was analyzed, taking into account twelve independent samples per treatment (three per each experimental unit). Total soluble solids (TSS) of grapes were measured by refractometry (Atago RX-5000, Tokyo, Japan). The pH and titratable acidity (TA) of the must were analyzed using an automatic titrator (Metrohm mod. 686, Herisau, Switzerland). Malic and tartaric acids were evaluated by using enzymatic kits from Boehringer Mannheim GmbH (Mannhein, Germany).

4. Microvinifications

After harvest, the grapes were maintained in the winery in a cold room in order to chill the grapes and obtain a homogeneous temperature of 4 C. Then, the grapes were destemmed, crushed, and distributed into 100-L tanks, one for each experimental unit. Later, $K_2S_2O_5$ per 100 kg of grapes was added and total acidity was corrected to 6 g/L. The selected yeast was added (10 g dry yeast/100 kg grapes; Laffort, DSM, Servian, France) and the alcoholic fermentation was carried out at 25 ºC. The cap was punched down three times a day and the must temperature and density were recorded during all the vinification process (around 14 days). At the end of the alcoholic fermentation, the wines were sampled for further analysis.

TABLE 1. Control and saline deficit irrigation treatments applied to Monastrell grapevines during three consecutive vintages (2016-2018).

Treatment	Irrigation start	Salts applied	EC (dS/m)	Irrigation (mm)		
				2016	2017	2018
Control	Ψs < -0.8 MPa	No salts added	1.8	116	104	104
Sul	Ψs < -0.8 MPa	Sulfates (Na_2SO_4 + $MgSO_4$)	5.0	118	106	102
Chl	Ψs < -0.8 MPa	Chlorides (NaCl)	5.0	114	102	102

EC: electrical conductivity. Ψs: stem water potential. Sul: water control + sulfates. Chl: water control + chlorides

5. Determination of anthocyanin in grapes and wines

At the time of reception of the grapes in the winery, a representative sample of 20 berries was selected for each experimental unit and was frozen at -20 °C for further analysis. The grapes were peeled with a scalpel and the skins were placed on absorbent paper to remove excess moisture. The skins were crushed in a mill (Vibratory Ball Mill Pulverisette, Cryo-box, Fritsch, Fischbach, Germany) and 2 g underwent extraction in 40 mL of MeOH (HPLC grade, PAI-ACS, Panreac, Barcelona, Spain) for 4 h with stirring (Labotron, INFORS, Bottmingen, Switzerland) at 150 rpm and at a temperature of 25 C. The methanolic extract was passed through a 0.45 µm nylon filter (Albet LabScience, Dassel, Germany) and analysed by high performance liquid chromatography (HPLC). Anthocyanin separation was carried out in a Waters 2960 liquid chromatograph (Waters, PA, USA), equipped with a Waters 996 diode-array detector (DAD). A reversed phase C-18 column of 25 x 0.4 cm, 100 Å (Primisep B2 SIELC Technologies, IL, USA) and 5 µm particle size was used. The analysis was performed at a temperature of 25 °C and the injected sample volume was 20 µL. The separation was carried out in a binary gradient, using as mobile phases a mixture of formic acid (4.5 %) (solvent A) and acetonitrile (solvent B), with a flow of 0.8 mL/min. Anthocyanin identification was carried out by comparing their spectrum with those reported in the literature (Revilla *et al.*, 1999; Castillo-Muñoz *et al.*, 2009), and they were confirmed by HPLC-mass (HPLC-MS) in a liquid chromatograph coupled to an LC-MSD-trap VL-01036 mass detector (Agilent Technologies, Waldbronn, Germany) equipped with an electrospray ionisation system (ESI). Anthocyanins were quantified at 520 nm using malvidin-3*O*-glucoside chloride as the external standard (Extrasynthése, Genay, France) (Bautista-Ortín, 2005). A calibration line were prepared using different concentrations of these compounds dissolved in the model wines.

Wine anthocyanin and vitisins analyses were carried out by direct injection of the previously filtered (0.45 µm nylon filter, Albet LabScience, Dassel, Germany) sample (wine) in a Waters 2690 liquid chromatograph (Waters) equipped with a Waters 996 diode-array detector (DAD). The column used was a CORTECS® Shield RP18 (Crawford Scientific, Strathaven, UK), 150 x 0.46 mm, and 2.7 µm in particle size. The volume of the injected sample was 20 µL and the sample was analysed at room temperature. The mobile phases used were: water with 4.5 % formic acid (solvent A) and acetonitrile (solvent B), with a flow of 0.90 mL/min. Both the identification and the quantification of anthocyanins were performed as previously described for grape skins.

6. Determination of tannin in grapes and wines

The tanins in the grapes and wines were analysed as described by Kennedy and Jones (2001). The method described hereafter is based on the phloroglucinolysis reaction which causes the polymeric tannin chains to break. Ten berries per replicate were selected. Both the seeds and

skins were separated from the grape pulp by using a scalpel and frozen at -20 °C for subsequent separate analysis. The tannin extraction was carried out with 10 mL of acetone/water (2:1, v/v) in an automatic orbital shaker (Labotron, Bottmingen, Switzerland) at 200 rpm for 24 h. The extract was then concentrated under reduced pressure at 35 °C by using a Centrivap concentrator (Labconco, Kansas city, USA) and was subsequently redissolved in 2 mL of MeOH.100 µL of phloroglucinol reagent (solution with 0.2N HCl in MeOH, 100 g/L of phloroglucinol and 20 g/L of ascorbic acid) was added to 100 µL aliquot of this methanolic extract; the subsequent phloroglucinolysis reaction was carried out in a warm water bath (Selecta, Unitronic OR) at 45 °C for 25 min and was stopped by adding 200 µL of 200 mM sodium acetate. Finally, the sample was centrifuged at 13000 rpm for 5 min and the obtained sample was analysed by high-performance liquid chromatography. A liquid chromatograph (Waters Model 2960, Philadelphia, USA), equipped with a Waters 996 diode-array detector, and an Atlantis dC18 reversed-phase column (Waters,) were used for quantification. A binary gradient of water and 2 % formic acid (solvent A) and acetonitrile/water/formic acid (80:18:2) (solvent B) was applied with a flow rate of 1 mL/min. Elution began at 0 % B for 5 min, followed by a linear gradient of 0 to 10 % B for 30 min and then a gradient of 10 to 20 % for 30 min, and finally the column was washed and re-equilibrated. The eluted peaks were monitored and identified at 280 nm. The monomeric flavan-3-ols (-)-epicatechin, (+)-catechin and (-)-epicatechin-3-*O*-gallate were identified by comparing their UV-Vis spectra with those of the pure standards (Sigma-Aldrich, Misuri, USA). The rest of the compounds were identified by comparing their spectra with those indicated in the literature (Kennedy *et al.*, 2001).

For the wine samples, the method proposed by Pastor del Río and Kennedy (2006) was used with certain modifications. The experimental procedure was as follows: 5 mL of wine was evaporated in a Centrivap concentrator (Labconco, Kansas City, USA). The dry extract was redissolved in 3 mL of water and passed through a SEP-PAK C18 cartridge (Vac 6 cm³/g) (Waters, Mildford, USA), previously activated with 10 mL of MeOH followed by 10 mL of water. The dry extract was washed with 20 mL of water, and then the targeted compounds were eluted with 10 mL of MeOH. The obtained extract was re-evaporated in the concentrator to finally redissolve the residue in 1 mL of MeOH. The tannins were identified and quantified in the methanolic extract as described for grapes.

7. Determination of stilbenes in grapes and wines

The extraction method used was that described by Bavaresco *et al.* (2001) with some modifications. Briefly, the skin from 20 g of berries was frosted and then lyophilised (Cryodos, Telstar, France). Samples (0.25 g) of lyophilised skin were extracted twice using 5 mL of Na_2CO_3 (5 %) and 5 mL of ethyl acetate in a liquid-liquid extraction. After that, the samples were homogenised using an Ultraturrax T-25 (Janke & Kunkel, Ika-Labortechnick, Breisgau, Germany)

and stirred at 200 rpm for 20 min (Labotron, INFORS, Switzerland). Once homogenised, the solutions were centrifuged in an Eppendorf 5810 R (Eppendorf, Hamburg, Germany) at 5000 rpm at 4 C for 5 min. The organic phase was dried in a Centrivap concentrator (Labconco, Kansas City, MO, USA) and the samples were re-diluted in 2 mL MeOH and filtered through a nylon 0.45 µm filter (Albet LabScience, Dassel, Germany). The samples were maintained in darkness and at low temperature (wrapped in foil and immersed in an ice bath) throughout the extraction process.

For the wine analysis, the same process as for the grapes was applied, but using 5 mL of wine instead of grape skins. Stilbenes were identified and quantified by HPLC-DAD (Alliance,Waters, Milford, EEUU). The methodology followed was that described by Guerrero *et al*. (2010) with some modifications. The samples were analysed using a Waters 2695 system (Waters). The analysis was carried out at a temperature of 30 C and the injected sample volume was 20 µL. Separation was performed on a Lichro Cart RP-18 column (Merck, Darmstadt, Germany), 25 × 0.4 cm, 5 µm particle size, using water and 5 % of formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min. The linear solvents gradient for the stilbenes analysis was as follows: 0 % B for 0 min; 15 % B for 15 min; 20% B for 40 min; 55 % B for 30 min and 0 B for 32 min. Chromatograms were recorded at 306 nm. The compounds were identified by comparing their UV spectra, which had been recorded with the diode array detector, with those reported in the literature. The stilbenes were quantified using a standard for each identified compound (piceid and resveratrol). The calibration lines were prepared using different concentrations of these compounds dissolved in the model wines.

8. Evaluation of the Antioxidant Capacity

To achieve a more reliable characterisation of the antioxidant capacity of the wines (2017 and 2018 vintages), three different spectrophotometric assays were applied: ABTS, DPPH, and FRAP. An automated microplate reader was used in all cases (FLUOstar Optima, BMG LabTech, France). In the cases of ABTS and FRAP, modified versions of the original antioxidant capacity assays were performed to fit these analyses in 96-well microplates, according to the procedures described by González-Centeno *et al*. (2012). For the DPPH assay, the analysis was carried out by adapting the methodology described by Vignault *et al*. (2018).

In all cases, the absorbance was determined at 25 ºC, and Trolox (0–1.3 mM) was used as a standard for the calibration curves. Diluted wine solutions were prepared at a ratio of 1:20 using distilled water. Antioxidant capacity was expressed as a mean of six determinations in mmols of Trolox equivalents per liter of wine (mmol Trolox).

9. Sensory analysis

A descriptive analysis was carried out with a panel of unqualified tasters (final consumers), in which three wines were tasted - one for each treatment. The different repetitions obtained for each treatment (four) were previously tasted to check that they were free of defects; any defected wines were discarded, and the remaining wines were mixed to obtain one wine per treatment. Colour, aroma, mouthfeel and overall value were each evaluated with scores ranging from 0 to 10, whereby 0 = the lowest intensity and 10 = the highest intensity. Wines from the 2017 vintage could not undergo a sensory evaluation, because a large number of them did not reach minimum quality scores to be able to perform the sensory analysis.

10. Data statistical analysis

Significant differences were assessed using two-way analysis of variance (ANOVA) (treatment x vintage). As the interaction *p*-value was not statistically significant for any of the studied chemical parameters, a one-way ANOVA was run individually for data corresponding to each significant factor. Then, Duncan´s Multiple Range test was applied to evaluate the differences, which were considered statistically significant at $p \leq 0.05$. The software used was SPSS Statistics 26 (IBM, Armonk, New York, USA). In order to find linear relationships between treatments for grape variables and wine variables, a multivariate discriminant analysis was performed for both grape and wine data by using Statgraphics 19 (Statgraphics Technologies, Inc., The Plains, Virginia, USA).

RESULTS AND DISCUSSION

1. Berry size and fruit composition

Significant differences ($p \leq 0.05$) in berry weight were only observed in 2018. Specifically, berry size was reduced in vines with Chl treatment compared to both Control and Sul treatments (Table 2). The trend observed in the previous vintages was a reduction in berry size when Sul and Chl saline treatments were applied, although the differences compared to the Control were not significant ($p > 0.05$). This reduction in berry size due to irrigation with saline water has been recently observed in the literature by Suarez *et al*. (2019), who reported that salinity treatments produced slight yield losses until EC 3.5 dS/m, after which, with increasing salinity, a very rapid decline in yield occurred. In a 5-year trial, Dag *et al*. (2015) also observed a slight yield decrease for vines irrigated with saline water (4.2 dS/m). Overall, the present results show a considerable vintage effect on berry size, even though the interaction between the two studied factors (treatment x year) was not significant (Table 2).

In terms of grape quality parameters (i.e., total soluble solids (TSS), pH, total acidity (TA), and tartaric and malic acid) no significant differences ($p > 0.05$) were observed among treatments (Table 2). It is worth noting that 1103 Paulsen (rootstock used in this study) has been described as a salt tolerant rootstock (Keller, 2015). It is considered to be able to reduce salt accumulation in its plant tissues (Walker *et al*., 2010), buffering the potential detrimental effects of phytotoxic ion build up on the vine tissues and improving the fruit quality parameters. In two out of the three studied vintages, a TSS increase was observed with salinity treatment compared to Control. This result is in agreement with those obtained by Ahmad (2016), who reported that the

TABLE 2. Quality parameters of Monastrell musts at harvest during three consecutive vintages (2016-2018).

Vintage	2016			2017			2018						
Treatment	Control	Sul	Chl	Control	Sul	Chl	Control	Sul	Chl	ANOVA	Treatment	Vintage	VxT
Berry weight (g)	1.5	1.3	1.3	1.8	1.6	1.7	1.7 b	1.6 b	1.4 a	**	ns	***	ns
TSS (ºBrix)	27.6	28.1	28.9	30.7	28.1	28.1	19.3	20.5	20.1	ns	ns	***	ns
pH	3.4	3.5	3.4	3.2	3.2	3.2	3.3	4.0	3.7	ns	ns	ns	ns
Total acidity (g/L)	6.6	6.6	6.7	9.8	9.6	9.3	5.6	6.5	6.4	ns	ns	***	ns
Tartaric acid (g/L)	4.3	4.3	4.2	4.9	4.7	5.0	4.8	4.3	4.7	ns	ns	***	ns
Malic acid (g/L)	2.1	2.0	2.3	3.8	3.5	3.4	2.2	2.2	1.9	ns	ns	***	ns

TSS: Total soluble solids. TA: total acidity. Sul: water control + sulfates. Chl: water control + chlorides. For each year and parameter, means followed by different letters indicate significant differences between treatments ($p \leq 0.05$) according to Duncan's multiple range test. *, ** and ***: indicate significant treatment effects at $p \leq 0.05$, 0.01 and 0.001, respectively. ns: indicates non-significant effects.

use of salt tolerant rootstocks (1103P, Sant creek and Freedom) for table grape under saline conditions was very effective and TSS accumulation was stimulated. Moreover, as in the present results, this author did not find any salinity effect on TA when 1103P rootstock was used. Once again, a significant vintage effect was observed on the fruit quality parameters and the interaction between treatment and year was not significant (Table 2). Of note are the high differences in TSS between vintages. In 2018, the lowest TSS concentration was found, probably due to the fact that this vintage represented the highest grape yield (Keller, 2015).

Grapevines are regarded as moderately sensitive to salinity. Salinity affects wine grape production through both osmotic and ionic processes. The effect of increasing salinity is first observed by a reduction in vine growth, followed by a decline in vine yield if saline conditions persist. Nevertheless, the effects of salinity on berry or juice composition seem to depend on the combination of cultivar and rootstock and on the salt concentration in the irrigation water, as well as on its time of application over the growing season (Mirás-Avalos and Intrigliolo, 2017).

2. Anthocyanins in grapes and wines

It is well known that anthocyanins are the main compounds responsible for the colour of red wines, playing a fundamental role in both colour stabilisation and antioxidant capacity (Li, 2006). In *Vitis vinifera*, monoglycoside anthocyanins and acylated monoglycoside anthocyanins have been identified in different proportions depending on the variety (Keller, 2015). Monastrell shows a high concentration of anthocyanins in grape skin, with a high percentage of non-acylated anthocyanins (70-89% of total anthocyanins) and a low percentage of anthocyanins linked to acetic, coumaric or caffeic acids (acylared anthocyanins) (Gil-Muñoz *et al.*, 2010).

The concentration of anthocyanin in Monastrell grapes at harvest is shown in Table 3. There were no significant differences ($p > 0.05$) between treatments for any of the studied vintages, probably due to the high variability shown in the replicates. Meanwhile, it should be noted that Sul treatment tended to increase the concentration

of all individual anthocyanins in grapes for all the studied vintages (Table 3). Nonetheless, for the same treatment, anthocyanin concentration in grapes differed significantly ($p \leq 0.05$) depending on the year (Table 3). Therefore, the grape anthocyanin concentration seems to depend more on environmental conditions - such as light exposure (He *et al.*, 2010), temperature (Tarara *et al.*, 2008), water status (Castellarin *et al.*, 2007) and viticultural practice (Keller, 2010) than on irrigation treatments. In fact, there are numerous studies about the effect of environmental factors on the synthesis and accumulation of anthocyanins (He *et al.*, 2010; Chorti *et al.*, 2010). However, to the best of our knowledge, no work focuses on the effects of irrigation using saline water on the concentration of anthocyanins in the grape. The effects of salinity on berries depends not only on the concentration of salt in the irrigation water, but also on the combination of cultivar and rootstock, as well as the time period of irrigation over the growing season.

Similar to the results for grapes, the applied irrigation treatments did not lead to any significant differences in terms of wine anthocyanin concentration, regardless of the vintage (Table 3). However, a significant vintage effect (linked to weather conditions) was observed on the concentration of total and individual anthocyanins. As expected, for the three studied years, the concentration of non-acylated anthocyanins in wine was significantly higher than that of acylated anthocyanins (Table 3) (García-Beneytez *et al.*, 2003). The trend of increasing anthocyanin level in berries for Sul was not observed in wines. These results agree with those of Walker *et al.* (2000), who did not find significant differences in total anthocyanin concentration of wines made from grapes irrigated with saline water.

Vitisins, which are pigments derived from low molecular weight anthocyanins formed as secondary metabolites of both alcoholic and malolactic fermentations (Alcalde-Eon, 2008), were also analysed in wines. In our study, salinity treatments did not lead to any significant differences in the concentration of vitisins in the wines compared to Control from the three studied vintages (Table 3). However, the concentration of vitisins found in grapes

TABLE 3. Concentration of anthocyanin in Monastrell grapes (mg/kg) at harvest and wines (mg/L) at the end of alcoholic fermentation during three consecutive vintages (2016-2018).

Treatment	Vintage	Grapes							Wines							
		Non acylated	TriOH	DiOH	Acylated	Acetates	Coumarates	Total	Non acylated	TriOH	DiOH	Acylated	Acetates	Coumarates	Vitisins	Total
Control	2016	526	405	120	108	25	79	634	445	358	87	121	32	55	17	598
Sul		625	479	169	133	32	96	759	445	357	87	119	31	50	17	582
Chl		548	379	146	117	27	86	666	424	340	83	122	32	52	17	566
Control	2017	408	295	112	94	18	70	502	196	149	47	96	17	40	9	302
Sul		482	359	123	101	21	76	584	218	170	48	81	15	34	7	308
Chl		417	310	107	98	19	75	516	233	183	50	81	13	34	8	323
Control	2018	230	187	43	104	14	80	334	195	176	19	41	8	29	2	239
Sul		294	228	65	121	17	94	416	199	180	19	41	8	29	2	243
Chl		204	155	48	98	15	75	302	197	178	19	44	9	31	2	243
Treatment		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vintage		***	***	***	ns	***	ns	***	***	***	***	ns	***	ns	***	***
Treatment x Vintage		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

TriOH: tri-hydoxylated anthocyanins. DiOH: di- hydroxylated anthocyanins. Sul: water control + sulfates. Chl: water control + chlorides. For each year and parameter, means followed by different letters indicate significant differences between treatments ($p \leq 0.05$) according to Duncan's multiple range test. *, ** and ***: indicate significant treatment effects at $p \leq 0.05$, 0.01 and 0.001, respectively. ns: indicates non-significant effects.

from 2016 was almost 2-fold that of 2017 and more than 8-fold that of 2018. It is well known that vitisins are formed by the cycloaddition of a molecule of pyruvic acid (vitisin A) or acetaldehyde (vitisin B) on an anthocyanin (Jones *et al.*, 2003). Therefore, anthocyanins being the precursors of vitisins synthesis, the differences observed in vitisins content in wines among vintages may be directly explained by the lower average concentration of anthocyanins in 2017 (311 mg/L) and 2018 (242 mg/L) compared to 2016 (583 mg/L).

3. Tannins in grapes and wines

Tannins, together with anthocyanins, are the molecules responsible for most of the sensory and organoleptic characteristics of red wines. Specifically, tannins are responsible for stabilising the colour and sensory characteristics of wines, such as astringency and bitterness (Chira *et al.*, 2009). Seeds usually contain a higher concentration of tannins and a greater proportion of galloylated units (% Gal) than skins, whereas skin tannins have a higher mean degree of polymerisation (mDP) (Gil, 2017).

The tannin concentrations of the seeds and skin of Monastrell grapes at harvest are shown in Tables 4 and 5 respectively. The tannin values for both seeds and skin were slightly higher when the salinity treatments (Sul and Chl) were applied, but this increase was not statistically significant ($p > 0.05$) for any of the studied vintages. These results are in agreement with those of Walker *et al.* (2000), who did not found significant differences in total polyphenol and total tannin content of Shiraz wines from vines irrigated with saline water. In contrast, for other plant species, such as *Leucaena leucocephala* and *Prosopis chilensis*,

it has been observed that irrigation with saline water increases the total concentration of condensed tannins (Taghried, 2012). In 2018, the Chl treatment significantly increased the concentration of epigallocatechin with respect to the other two treatments. Although these differences have only been observed in the last of the three years of the study (probably due to a cumulative effect), this increase could be due to the specific effect of Cl- in the phenylpropanoid pathway, which is responsible for the synthesis of different classes of metabolites, such as lignins, flavonoids and stilbenoids (Carvalho *et al.*, 2015). Moreover, the phenylpropanoid biosynthetic pathway is normally stimulated by salt stress, resulting in an increase in the production of different phenolic compounds (Sharma *et al.*, 2019).

Both the mean degree of polymerisation (mDP) and the percentage of galloylation (% Gal) (Tables 4 and 5) were also evaluated. These two parameters are related to the level of astringency and bitterness of wines. No significant differences ($p > 0.05$) among treatments were found for mDP, nor for % Gal; therefore, irrigation with saline water (Chl and Sul treatments) do not seem to have an impact on the sensory quality (astringency and bitterness) of the wines. According to the literature (González-Centeno, 2013), mDP values for the grape skin show high variability (0.85-85.7). The mDP values for skins and seeds obtained in this study are within the ranges previously established in the bibliography, and are very similar to those obtained by (Moreno-Pérez *et al.*, 2013) for the Monastrell variety in the same study area (Jumilla). This high mDP variability observed between different varieties, or even within the same grape varieties, occurs in response to changing environmental factors (Kennedy *et al.*, 2001; Kennedy *et al.*, 2002; Downey *et al.*, 2003). Total tannin and extension units in

TABLE 4. Concentration of tannin in seeds (mg/L) of Monastrell grapes at harvest during three consecutive vintages (2016- 2018).

Treatment	Vintage	mDP	% Gal	CAT-E	EPI-E	CAT-T	EC3OG-E	EPI-T	EC3OG-T	µg/g skin	µg/berry	mg/kg
Control		6.4	15.9	203	1615	135	478	169	103	35519	2741	1303
Sul	2016	6.5	16.3	179	1326	116	398	122	121	38256	2264	1387
Chl		6.5	15.8	211	1508	133	450	147	123	33821	2575	1526
Control		7.3	13.6	296	2346	178	618	235	102	49045	3778	1844
Sul	2017	7.8	14.1	274	2237	164	609	204	121	61972	3610	2259
Chl		7.3	14.1	247	2002	160	517	159	128	42560	3227	1916
Control		7.4	15.4	185	1517	100	442	159	104	31559	2510	1190
Sul	2018	7.4	16.0	195	1574	111	481	160	111	24766	2635	1443
Chl		6.7	16.0	193	1823	129	562	203	127	38664	3040	1730
Treatment		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vintage		*	***	***	***	*	*	ns	ns	*	*	***
Treatment x Vintage		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

mDP: mean degree of polymerization. % Gal: percentage of galloylation. CAT E: extension (+)-catechin. EPI E: extension (-)-epicatechin. CAT T: terminal (+)-catechin. E3OG E: extension (-)-epicatechin 3-O-gallate. EPI T: terminal (-)-epicatechin. E3OG T: terminal (-)-epicatechin 3-O-gallate. Sul: water control + sulfates. Chl: water control + chlorides. For each year and parameter, means followed by different letters indicate significant differences between treatments ($p \leq 0.05$) according to Duncan's multiple range test. *, ** and ***: indicate significant treatment effects at $p \leq 0.05$, 0.01 and 0.001, respectively. ns: indicates non-significant effects.

TABLE 5. Concentration of tannin in skins (mg/L) of Monastrell grapes at harvest during three consecutive vintages (2016-2018).

Treatment	Vintage	mDP	% Gal	EGC-E	CAT-E	EPI-E	CAT-T	EC3OG-E	EPI-T	EC3OG-T	µg/g skin	µg/berry	mg/kg
Control	2016	16.6	1.1	240	11	485	29	22	19	nd	5047	808	394
Sul		14.8	2.0	205	11	441	30	30	18	nd	5988	738	455
Chl		14.7	1.9	228	12	494	30	28	24	0.14	6803	819	487
Control	2017	14.1	0.8	172	7	481	38	9	11	0.18	4583	720	349
Sul		14.3	1.0	170	7	485	38	10	12	1.20	6036	725	452
Chl		15.1	1.0	176	7	464	33	10	11	0.51	5782	705	414
Control	2018	15.0	2.3	**214 a**	14	518	33	29	20	nd	5746	829	396
Sul		14.9	2.7	**223 a**	14	498	32	30	19	3.21	6675	819	441
Chl		14.9	2.2	**283 b**	16	575	38	30	24	1.57	6849	970	552
Treatment		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vintage		**	***	**	***	ns	*	***	*	ns	ns	*	ns
Treatment x Vintage		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

mDP: mean degree of polymerization. % Gal: galloylation. CAT E: extension (+)-catechin. EPI E: extension (-)-epicatechin. E3OG E: extension (-)-epicatechin 3-O-gallate. EPI T: terminal (-)-epicatechin. E3OG T: terminal (-)-epicatechin 3-O-gallate. EGC E: extension epigallocatechin. Sul: water control + sulfates. Chl: water control + chlorides. For each year and parameter, means followed by different letters indicate significant differences between treatments (p ≤ 0.05) according to Duncan's multiple range test. *, ** and ***: indicate significant treatment effects at p ≤ 0.05, 0.01 and 0.001, respectively. ns: indicates non-significant effects. Different letters a-b means significant differences (p ≤ 0.05) among irrigation treatments for the same vintage.

TABLE 6. Concentration of tannin (mg/L) in Monastrell wines at the end of alcoholic fermentation during three consecutive vintages (2016-2018).

Treatment	Vintage	mDP	% Gal	EGC-E	CAT-E	EPI-E	CAT-T	EC3OG-E	EC3OG-T	EPI-T	Total tannin
Control		10.8	7.9	106	42	478	18	39	47	16	697
Sul	2016	10.2	8.2	121	47	510	21	42	56	19	819
Chl		10.0	8.3	128	49	541	20	45	59	21	867
Control		4.7	4.1	58	31	207	46	16	6	28	389
Sul	2017	4.9	3.9	52	26	225	48	15	4	28	396
Chl		4.1	4.1	61	28	177	50	18	41	28	348
Control		4.3	4.2	78 a	8	163	40	10	10	28	340
Sul	2018	4.5	4.8	78 a	9	152	36	10	12	26	313
Chl		4.0	5.1	83 b	8	136	36	10	11	30	305
ANOVA		ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Treatment		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vintage		***	***	***	***	***	***	***	***	***	***
Treatment x Vintage		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

mDP: mean degree of polymerization. %Gal: galloylation. CAT E: (+)-catechin. EPI E: (-)-epicatechin. CAT T: terminal (+)-catechin. E3OG E: extension (-)-epicatechin 3-O-gallate. EPI T: terminal (-)-epicatechin. E3OG T: terminal (-)-epicatechin 3-O-gallate. EGC E: extension epigallocatechin. Sul: water control + sulfates. Chl: water control + chlorides. For each year and parameter, means followed by different letters indicate significant differences between treatments ($p \leq 0.05$) according to Duncan's multiple range test. *, ** and ***: indicate significant treatment effects at $p \leq 0.05$, 0.01 and 0.001, respectively. ns: indicates non-significant effects. Different letters a-b means significant differences ($p \leq 0.05$) among irrigation treatments for the same vintage.

wines are depicted in Table 6. As observed in the case of grapes (skins and seeds), no significant statistical differences ($p > 0.05$) were found among treatments for total tannin, mDP, % Gal and extension units (except for the epigallocatechin extension [EGC-E] in 2018).

4. Stilbenes in grapes and wines

The results obtained for the stilbenes composition of Monastrell grapes and wines are shown in Table 7. Once again, in terms of individual and total stilbenes, no significant differences ($p > 0.05$) were observed among treatments for grapes or wines in any of the three years of study. In the case of grapes, piceid stilbenes content was greater than that of resveratrol, regardless of the vintage. This is due to the fact that Monastrell grapes preferentially accumulate stilbenes in their glucosylated form, with piceid present in high concentrations (Ruiz-García *et al.*, 2013). The high variability obtained in the analysis of stilbenes in grapes, even within the same grape variety (Gatto *et al.*, 2008), may explain the lack of statistical differences among the experimental treatments. This variability is due to the low concentration of stilbenes in the skins and the many factors on which their synthesis depends (Bavaresco *et al.*, 2016).

In the case of wines, the weather conditions had a significant effect on the content of both *cis*- and *trans*-piceid, both *cis*- and *trans*-resveratrol and total stilbenes. Specifically,

the total concentration of stilbenes in the wines was greater in the 2017 vintage, regardless of the treatment (Table 7). The higher percentage of *Botrytis cinerea* fungus present on the grapes that year might be the reason for these differences between vintages (Negri *et al.*, 2017).

Stilbene biosynthesis is influenced by a wide array of abiotic environmental factors. As well as light, temperature and drought, published reviews have identified salinity as an important abiotic stress source able to modulate stilbene biosynthesis (Valletta *et al.*, 2021). Salt stress is thus an important constraint to plant development processes and physiological homeostasis, causing ROS induction and perturbations to photosynthesis (Souid *et al.*, 2019). A recent experiment on *Vitis vinifera* plants subjected to a NaCl treatment revealed that the salt-tolerant cv. Razegui did not show high variations in stilbenes biosynthesis, while the salt-sensitive cv. Syrah showed an increase in content of *trans*-resveratrol, *trans*- and *cis*-piceid- probably to cope with the higher oxidative disturbance (Souid *et al.*, 2019). This might explain the low variability in the total concentration of stilbenes in grape and wine among treatments in the present experiment, as the salinity tolerant rootstock, 1103P (*V. berlandieri* x *V. rupestris*), was used.

5. Antioxidant capacity

The antioxidant capacity of wines from both 2017 and 2018 vintages are shown in Table 8. The antioxidant potential of

TABLE 7. Concentration of stilbenes in Monastrell grapes (mg/kg) at harvest and wines (mg/L) at the end of alcoholic fermentation during three consecutive vintages (2016-2018).

Treatment	Year	Grapes					Wines				
		T-Piceid	C-Piceid	T-Resveratrol	C-Resveratrol	Total stilbenes	T-Piceid	C-Piceid	T-Resveratrol	C-Resveratrol	Total stilbenes
Control	2016	1.7	1.0	0.2	0.7	3.7	3.3	5.7	4.0	1.2	14.3
Sul		1.9	0.8	0.4	0.2	3.4	3.0	5.8	3.1	1.3	13.3
Chl		1.9	1.1	0.4	0.2	3.6	3.0	5.6	3.6	1.4	13.7
Control	2017	0.7	2.8	0.5	0.6	4.2	4.7	1.8	11.6	4.5	23.2
Sul		0.7	2.8	0.6	0.5	4.4	4.1	1.5	12.3	4.8	22.9
Chl		0.7	2.6	0.6	0.6	4.3	3.8	1.7	9.6	3.5	19.7
Control	2018	0.7	0.9	0.2	0.1	2.0	3.3	1.2	7.8	0.1	12.7
Sul		0.7	1.0	0.2	0.2	2.3	3.2	1.6	8.0	0.1	13.0
Chl		0.7	1.0	0.2	0.1	2.1	3.4	1.5	8.5	0.1	13.5
Treatment		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vintage		**	***	*	ns	ns	*	***	***	***	***
Treatment x Vintage		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Sul: water control + sulfates. Chl: water control + chlorides. T-piceid: Trans-piceid, C-Piceid: Cis-Piceid, T-Resveratrol: Trans-Resveratrol, C-Resveratrol: Cis-Resveratrol. For each year and parameter, means followed by different letters indicate significant differences between treatments ($p < 0.05$) according to Duncan's multiple range test. *, ** and ***: indicate significant treatment effects at $p \leq 0.05$, 0.01 and 0.001, respectively. ns: indicates non-significant effects.

TABLE 8. Antioxidant capacity results (mmol trolox equivalents) for wines from 2017 and 2018 vintages.

Treatment	Vintage	ABTS	DPPH	FRAP
Control		6.3	32.3	4.4
Sul	2017	6.7	33.3	4.4
Chl		5.8	34.1	4.4
Control		2.7	10.9	2.9
Sul	2018	2.1	11.0	2.7
Chl		2.5	11.1	2.6
Treatment		ns	ns	ns
Vintage		***	***	***
Treatment x Vintage		ns	ns	ns

ABTS: 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) , DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, FRAP: ferric reducing/ antioxidants power. Regardless of the analysis method, all antioxidant capacity results are expressed in mmol trolox equivalents. *, ** and ***: indicate significant treatment effects at $p \leq 0.05$, 0.01 and 0.001, respectively. ns: indicates non-significant effects.

Monastrell wines from different saline irrigation treatment was assessed as ABTS, DPPH, and FRAP. In agreement with the literature, wine phenolic composition, and therefore antioxidant capacity, is influenced by different factors: grape variety, vineyard location, cultivation system, climate and soil type (Shahidi and Naczk, 1995). However, it can be observed in the present assay that the interaction between treatment and vintage factors did not lead to any significant differences in antioxidant capacity among samples ($p > 0.05$). Likewise, according to the one–way ANOVA, the ABTS, DPPH or FRAP values of wines from the same vintage did not differ significantly ($p > 0.05$) from one irrigation treatment to another. Only the vintage factor seemed to have impacted the antioxidant capacity of wines, as previously observed by Majo *et al*. (2008). These authors observed significant differences in the antioxidant capacity of the wines from some red grape varieties, such as Cabernet-Sauvignon or Merlot, in three years of study (2002-2004). In any case, Ours antioxidant capacity results are in agreement with the bibliographic values obtained by ABTS to those obtained for the Monastrell variety cultivated in southeast of Spain (3.7-3.9 mmol/TE/mL), and higher than those obtained for DPPH (5.3-5.6 mmol/TE/mL) (Pérez-Álvarez *et al*., 2021).

6. Sensory analysis of wines

To determine whether the results obtained for the different saline irrigation treatments would be reflected in the wines at a sensory level, a descriptive test was performed for all the wines from the 2016 and 2018 vintages (Figure 1). Sensory analysis was not carried out on the wines from the 2017 vintage, because their alcoholic fermentation was not completed, as a consequence of the high brix degree of the musts.

The sensory analysis did not reveal any clear differences or preferences for different wines. The 2016 vintage wines (Figure 1a) from vines irrigated with saline water (Sul and Chl) were better evaluated by the panel of tasters
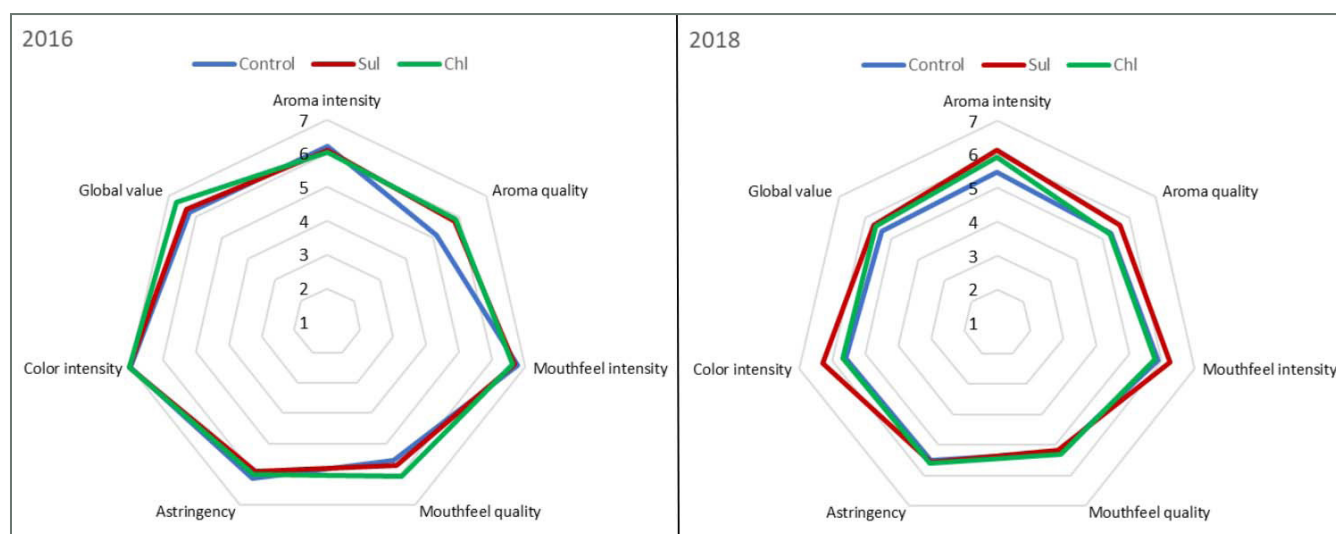

FIGURE 1. Descriptive sensory analysis of the wines from Control and salinity (Sul: water control + sulfates, Chl: water control + chlorides) treatments in a) 2016 and b) 2018 vintages.
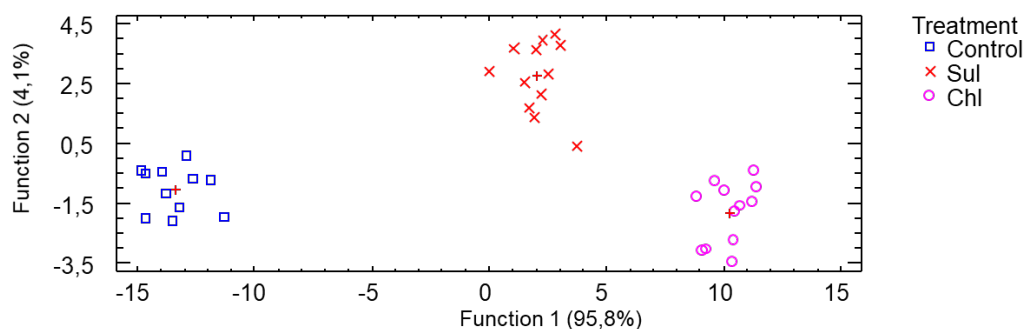
FIGURE 2. Distribution of grape samples in the two dimensional coordinated system by two first discriminant functions.
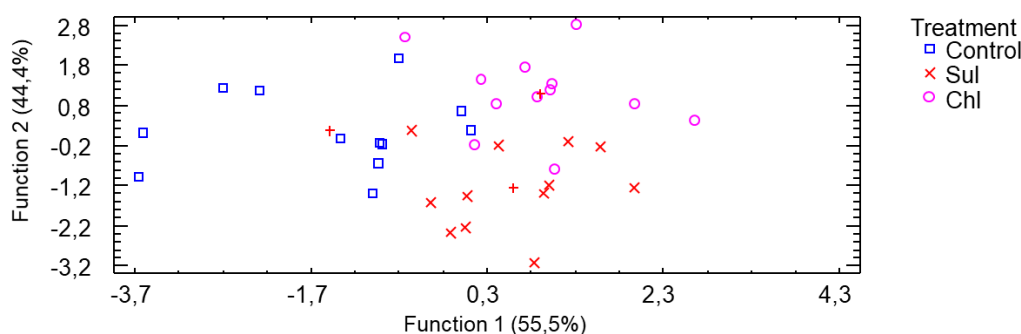

FIGURE 3. Distribution of wine samples in the two dimensional coordinated system by two first discriminant functions.

than vines from the control treatment in terms of both aroma and mouthfeel quality parameters, even if these differences were not significant ($p > 0.05$). In 2018 (Figure 1b), wines from vines which underwent the Sul treatment were the most appreciated in terms of aroma and mouthfeel intensity, aroma quality, colour intensity and they were given the highest overall rating. In general, the wines from the grapes irrigated with saline water were the most appreciated by the panel for both vintages. These results are in agreement with those observed by Scacco *et al.* (2010) in a saline irrigation study on wines from the Nero d'Avola variety; they observed that the increase in salinity in the soil enhanced colour intensity and the purple tones of the wines, and improved their saline, citrus and fruity aromas. In that research, wines from medium-high saline soil were preferred by the tasters.

It is important to note that the increased salinity in grapes and wine is a phenomenon associated with semiarid and arid regions, such as the Mediterranean viticultural area. In addition, salinity derived attributes, such as brackish, seawater and soapy, are aromas considered as negative and have been correlated with high concentrations of Na, K and Cl in wines (Mira de Orduña, 2010).

7. Discriminant analysis

The linear discriminant analysis was conducted using grape variables (Figure 2) and wine variables (Figure 3) separately. In the case of grapes, 33 variables were used and two discriminant functions (function 1 [95.8 %] and function 2 [4.1 %]) were obtained. These discriminant

functions allowed 100 % of the grape samples to be correctly classified. Figure 2 shows three different groups clearly separated according to the irrigation treatment (Control, Sul and Chl). For function 1, the Control samples are totally separated from the saline treatment (Sul and Chl) samples, which appear closer, but are clearly differentiated due to function 2, on the right side of the graph. The standardised coefficients of the discriminant functions (data not shown) showed that coumarates and epicatechin are the variables with the highest influence (both in a positive way) on the first and second functions respectively. In the case of wines, the discriminant analysis considered 24 variables (Figure 3) and two discriminant functions were also obtained (function 1 [55.5 %] and function 2 [44.4 %]). As can be observed, there are no clear differences among irrigation treatments.

Therefore, only grape parameters were able to discriminate among the irrigation treatments. This lack of discrimination according to wine variables is probably due to the dilution of grape differences throughout the winemaking process. All wines were vinified in the same way, which leads to a homogenisation of the final wines.

CONCLUSIONS

After three consecutive vintages (2016–2018) of saline deficit irrigation treatments in a commercial vineyard in Southeast of Spain, the phenolic composition of both grape and wine from Monastrell variety was analysed.

In general, the saline irrigation treatments did not have a significant effect on the oenological parameters (the content of anthocyanins, tannins and stilbenes) or on the results of the sensory analysis. The lack of significant differences between the irrigation treatments for the studied parameters was probably due to: i) the use of a salinity tolerant rootstock (1103P), ii) the sandy-loam soil, which favours the leaching of salts, iii) the high variability of the results obtained in the vineyard, and iv) the relatively low concentration of salts added to standard water to induce salt stress. Moreover, the potentially toxic salts (Na_2SO_4 + $MgSO_4$ and NaCl) were not found to have any effects on grape and/or wine parameters.

In summary, due to the lack of availability of good quality water for vineyard irrigation in certain arid and semiarid environments, the use of saline water with high electrical conductivity (5 dS/m) is a feasible way of dealing with water scarcity, with no further influence on the phenolic quality of grapes and their corresponding wines. This practice is possible as long as the vineyard soil has a texture that favours leaching, avoiding the accumulation of salts in the short to medium term. In any case, more research is needed to evaluate how the phenolic composition of both grapes and wines is influenced by a long-term saline irrigation in the vineyard.

ACKNOWLEDGEMENTS

REFERENCES

Ahmad, O.A. (2016). Salinity soil effects on yeild, fruit quality and mineral composition of superior seedless grapevines grafted on some rootstocks. *Applied of Science*, 16, 359-371. https://doi.org/10.3923/jas.2016.359.371

Alcalde-Eon, C. (2008). Anthocyanin-derived pigments originated during wine making and aging, and their contribution to wine colour. Ph. D. Thesis, University of Salamanca, Spain.

Bautista-Ortín, B. (2005). Técnicas enológicas para la obtención de vinos de Monastrell de alto contenido polifenólico. Doctoral Thesis. Universidad de Murcia, Spain. p. 346.

Bavaresco, L., Pezzutto, S., Ragga, A., Ferrari, F. & Trevisan, M. (2001). Effect of nitrogen supply on trans-resveratrol concentration in berries of *Vitis vinifera* L. cv. Cabernet-Sauvignon. *Vitis*, 40, 229-230.

Bavaresco, L., Luigi, L., Busconi, M., Flamini, R. & De Rosso, M. (2016). Wine resveratrol: from the ground up. Nutrients. *Journal of Agriculture and Food Chemistry*, 8, 222-228. https://doi.org/10.3390/nu8040222

Carvalho, L. C., Vidigal, P. & Amâncio, S. (2015). Oxidative stress homeostasis in grapevine (*Vitis vinifera* L.). *Frontiers in Environmental Science*, 3, 20. https://doi.org/10.3389/fenvs.2015.00020

Castellarin, S.D., Matthews, M.A., Di Gaspero, G. & Gambetta, G.A. (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta*, 227(1):101-12. https://doi.org/10.1007/s00425-007-0598-8

Castillo-Muñoz, N., Gomez-Alonso, S., Garcia-Romero, E., Gomez, M.V., Velders, A.H. & Hermosin-Gutierrez, I. (2009). Flavonol 3-O-Glycosides series of *Vitis vinifera* Cv. Petit Verdot red wine grapes. *Journal of Agricultural and Food Chemistry*. 57, 209-219. https://doi.org/10.1021/jf802863g

Chaves, M., Zarrouk, O., Francisco, R., Costa, J., Santos, T., Regalado, A., Rodrigues M. & Lopes, C. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Botany*, 105, 661–676. https://doi.org/10.1093/aob/mcq030

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. *Journal of Agricultural and Food Chemistry*, 57, 545–553. https://doi.org/10.1021/jf802301g

Chorti, E., Guidoni, S., Ferrandino, A. & Novello, V. (2010). Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. *American Journal of Enology and Viticulture*, 61: 23–30.

Dag, A., Ben-Gal, A., Goldberger, S., Yermiyahu, U., Zipori, I., Or, E., David, I., Netzer, Y. & Kerem, Z. (2015). Sodium and chloride distribution in grapevines as a function of rootstock and irrigation water salinity. *American Journal of Enology and Viticulture*, 66, 80-84. https://doi.org/10.5344/ajev.2014.14019

Dai, Z.W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.P. & Bordenave, L. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: A review. *American Journal of Enology and Viticulture*, 62, 413–425. https://doi.org/10.5344/ajev.2011.10116

Downey, M., Harvey, J. & Robinson, S. (2003). Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research*, 9, 15-27. https://doi.org/10.1111/j.1755-0238.2003.tb00228.x

García-Beneytez, E., Revilla, E. & Cabello, F. (2003). Anthocyanin pattern of several red grape cultivars and wines made from them. *European Food Research and Technology*, 215, 32-37. https://doi.org/10.1007/s00217-002-0526-x

Gambetta, G. A., Herrera, J. C., Dayer, S., Feng, Q., Hochberg, U. & Castellarin, S. D. (2020). The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *Journal of Experimental Botany*, 71(16), 4658–4676. https://doi.org/10.1093/jxb/eraa245

Gatto, P., Vrhovsek, U., Muth, J., Segala, C., Romualdi, C., Fontana, P., Pruefer, D., Stefanini, M., Moser, C., Mattivi, F. & Velasco, R. (2008). Ripening and genotype control stilbene accumulation in healthy grapes. *Journal of Agricultural and Food Chemistry*, 56, 773-785. https://doi.org/10.1021/jf8017707

Gil, R. (2017). Improving phenolic and chromatic characteristics of Monastrell, Merlot and Syrah wines by using methyl jasmonate and benzothiadiazole. *OENO One*, 51(1). https://doi.org/10.20870/oeno-one.2017.51.1.1814

Gil-Muñoz, R., Fernández-Fernández, J.I., Vila-López, R. & Martínez-Cutillas, A. (2010). Anthocyanin profile in Monastrell grapes in six different áreas from Denomination of Origen Jumilla during ripening stage. *International Journal of Food Science and Technology*, 45, 1870–1877. https://doi.org/10.1111/j.1365-2621.2010.02340.x

González-Centeno, M.R., Jourdes, M., Femenia, A., Simal, S., Rosselló, C. & Teissedre, P.L. (2012) Proanthocyanidin composition and antioxidant potential of the stem winemaking byproducts from 10 different grape varieties (*Vitis vinifera* L.). *Journal of Agricultural Food Chemistry*. 23101762. https://doi.org/10.1021/jf303047k

González-Centeno, M.R. (2013) Caracterización de los subproductos de la industria vitivinícola como fuente de fibra dietética y compuestos fenólicos. uso de los ultrasonidos de potencia para la extracción de la fracción fenólica. Universitat de les Illes Balears.

Guerrero, F., Puertas, B., Férnández, M., Palma, M. & Cantos-Villar, E. (2010). Induction of stilbenes in grapes by UV-C: Comparison of different subspecies of Vitis. *Innovative Food Science & Emerging Technologies*, 11, 231–238. https://doi.org/10.1016/j.ifset.2009.10.005

Hawker, J.S. and Walker, R.R. (1978). The effect of sodium chloride on the growth and fruiting of Cabernet-Sauvignon vines. *American Journal of Enology and Viticulture*, 29, 172-176.

He, F., Mu, L., Yan, G.-L., Liang, N.-N., Pan, Q.-H., Wang, J., Reeves, M.J. & Duan, C.Q. (2010). Biosynthesis of Anthocyanins and Their Regulation in Colored Grapes. *Molecules*, 15, 9057–9091. https://doi.org/10.3390/molecules15129057

Hepaksoy, S., Ben-Asher, J., De Malach, Y., David, I., Sagih, M. & Bravdo, B. (2007). Grapevine Irrigation with Saline Water: Effect of Rootstocks on Quality and Yield of Cabernet-Sauvignon. *Journal of Plant Nutrition*, 29:5, 783-795. https://doi.org/10.1080/01904160600649153

Hirzel, D.R., Steenwerth, K., Parikh, S.J. & Oberholster, A. (2017). Impact of winery wastewater irrigation on soil, grape and wine composition. *Agricultural Water Management*, 31, 178-189. https://doi.org/10.1016/j.agwat.2016.10.019

IPCC (2019). Climate Change 2019: Impacts, Adaptation, and Vulnerability. http://ipcc-wg2.gov/AR5/report/final-drafts.

Jones, G., Iland, P., Markides, A. & Asenstorfer, R. (2003) Formation of vitisins during ferementation and maduration. *Australian Journal of Grape and Wine Research*, 9, 40–46.

Keller, M. (2010). Managing grapevines to optimize fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research*, 16, 56–59. https://doi.org/10.1111/j.1755-0238.2009.00077.x

Keller, M. (2015). The science of Grapevines, Anatomy and Physiology. Second edition. Ed. Elsevier. San Diego, CA.

Kennedy, J. A. and Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry*, 49, 1740–1746. https://doi.org/10.1021/jf001030o

Kennedy, J.A., Matthews, M.A. & Waterhouse, A.L. (2002). Effect of maturity and vine water status on grape skin and wine flavonoids. *American Journal of Enology and Viticulture*, 53, 268-274.

Kennedy, J. A., Hayasaka, Y., Vidal, S., Waters, E. J. & Jones, G. P. (2001). Composition of grape skin proanthocyanidins at different stages of Berry development. *Journal of Agricultural and Food Chemistry*, 49, 5348–5355. https://doi.org/10.1021/jf010758h

Li, H. (2006). Wine Chemistry. Ed. China Science Press. Beijing, China.

López-Urrea, R., Montoro, a., Mañas, F., López-Fuster, P. & Fereres, E. (2012). Evapotranspiration and crop coefficients from lysimeter measurements of mature Tempranillo wine grapes. Agricultural Water Management, 112, 13–20. https://doi.org/10.1016/j.agwat.2012.05.009

Majo, D.D., Guardia, M.L., Giammanco, S., Neve, L.L. & Giammanco, M. (2008). The antioxidant capacity of red wine in relationship with its polyphenolic constituents. *Food Chemistry*, 111(1), 45–49. https://doi.org/10.1016/j.foodchem.2008.03.037

Mirás-Avalos, J.M. & Intrigliolo, D.S. (2017). Grape Composition under abiotic constrains: Water Stress and Salinity. *Frontiers in Plant Science*, 8, 851-872. https://doi.org/10.3389/fpls.2017.00851

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*, 43, 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

Moreno-Pérez, A., Férnandez-Férnandez, I., Bautista-Ortín, A.B. & Gómez-Plaza, E. (2013). Influence of winemaking techniques on proanthocyanidin extraction in Monastrell wines from four different areas. *European Food Research and Technology*. ISSN 1438-2377. https://doi.org/10.1007/s00217-012-1899-0

Negri, S., Lovato, A., Boscaini, F., Salvetti, E., Torriani, S., Commisso, M., Danzi, R., Ugliano, M., Polverari, A., Tornielli, G. B. & Guzzo, F. (2017). The Induction of Noble Rot (*Botrytis cinerea*) Infection during Postharvest Withering Changes the Metabolome of Grapevine Berries (*Vitis vinifera* L., cv. Garganega). *Frontiers in Plant Science*, 8, 1002. https://doi.org/10.3389/fpls.2017.01002

Netzer, Y., Shenker, M. & Schwartz, A. (2014). Effects of irrigation using treated wastewater on table grape vineyards: dynamics of sodium accumulation in soil and plant. *Irrigation Science*, 32, 283–294. https://doi.org/10.1007/s00271-014-0430-8

Pastor del Río, J.L. & Kennedy, J.A. (2006). Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *American Journal of Enology and Viticulture*, 57, 125–132.

Pérez-Álvarez, E. P., Intrigliolo, D.S., Almajano, M.P., Rubio-Bretón, P. & Garde-Cerdán, T. (2021). Effects of Water Deficit Irrigation on Phenolic Composition and Antioxidant Activity of Monastrell Grapes under Semiarid Conditions. *Antioxidants*, 10, 8: 130. https://doi.org/10.3390/antiox10081301

Posmyk, M.M., Kontek, R. & Janas, K.M. (2009). Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicology and Environmental Safety*, 72: 596-602. https://doi.org/10.1016/j.ecoenv.2008.04.024

Ranjan, P., Kazama, S., Sawamoto, M. & Sana, A. (2009). Global scale evaluation of coastal fresh groundwater resources. *Ocean Coast Management,* 52(3–4):197–206. https://doi.org/10.1016/j.ocecoaman.2008.09.006

Revilla, I., Perez-Magarino, S., Gonzalez-SanJose, M.L. & Beltran, S. (1999). Identification of anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode array and mass spectrometric detection. *Journal of Chromatogry*, 847, 83-90. https://doi.org/10.1016/S0021-9673(99)00256-3

Rice-Evans, C.A., Miller, N.J. & Paganaga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2: 152-159. https://doi.org/10.1016/S1360-1385(97)01018-2

Ruiz-García, Y., Romero-Cascales, I., Bautista-Ortín, A. B., Gil-Muñoz, R., Martínez-Cutillas,A. & Gómez-Plaza, E. (2013). Increasing bioactive phenolic compounds in grapes: Re-sponse of six Monastrell grape clones to benzothiadiazole and methyl jasmonatetreatments. *American Journal of Enology and Viticulture*, 64,459–465. https://doi.org/10.5344/ajev.2013.13038

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'. *Agricultural Water Management*, 98, 1171-1179. https://doi.org/10.1016/j.agwat.2011.02.011

Savoi, S., Herrera, J. C., Carlin, S., Lotti, C., Bucchetti, B., Peterlunger, E., Castellarin, S. D. & Mattivi, F. (2020). From grape berries to wines: drought impacts on key secondary metabolites. *OENO One*, 54(3), 569–582. https://doi.org/10.20870/oeno-one.2020.54.3.3093

Scacco, A., Verzera, A., Carmela, M., Lanza, A., Tripodi, G. & Dima, G. (2010). Influence of soil Salinity on sensory characteristics and volatile aroma compounds of Nero d'Avola Wine. *American*

Journal of Enology and Viticulture, 65, 498-505. https://doi.org/10.5344/ajev.2010.10003

Schultz, H.R. and Jones, G.V. (2010). Climate induced historic and future changes in viticulture. *Journal of Wine Research*, 21,137-145. https://doi.org/10.1080/09571264.2010.530098

Shahidi, F. and Naczk, M. (1995). Wine in food phenolics: Sources, chemistry, effects, applications. Pennsylvania: Technomic Publishing Co (pp. 136–148).

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. & Zheng, B. (2019). Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. *Molecules* (Basel, Switzerland), 24(13), 2452. https://doi.org/10.3390/molecules24132452

Souid, I., Toumi, I., Hermosín-Gutiérrez, I., Nasri, S., Mliki, A. & Ghorbel, A. (2019). The effect of salt stress on resveratrol and piceid accumulation in two *Vitis vinifera* L. cultivars. *Physiology and molecular biology of plants,* 25: 625–635. https://doi.org/10.1007/s12298-019-00668-2

Suarez, D. L., Celis, N., Anderson, R. G. & Sandhu, D. (2019). Grape Rootstock Response to Salinity, Water and Combined Salinity and Water Stresses. *Agronomy*, 9 (6), 321. https://doi.org/10.3390/agronomy9060321

Tarara, J.M., Lee, J., Spayd, S.E. & Scagel, C.F. (2008). Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. *American Journal of Enology and Viticulture*. 59:235–247

Taghried, E.L. (2012). Effect of Salinity on Tannins Content of Leucaena leucocephala (Lam.) de Wit. and Prosopis chilensis (Molina) Stuntz and Techniques for Their Reduction. JOUNAL Botany 2º international conference.

Valletta, A., Iozia, L. M. & Leonelli, F. (2021). Impact of Environmental Factors on Stilbene Biosynthesis. Plants (Basel, Switzerland), 10 º(1), 90. https://doi.org/10.3390/plants10010090

van Leeuwen, C., Tregoat, O., Choné, X., Bois, B., Pernet, D. & Gaudillére, J.P. (2009) Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *Journal International des Sciences de la Vigne et du Vin*, 43, 121-134. https://doi.org/10.20870/oeno-one.2009.43.3.798

Vignault, A., González-Centeno, M. R., Pascual, O., Gombau, J., Jourdes, M., Moine, V., Iturmendi, N., Canals, J. M., Zamora, F. & Teissedre, P.-L. (2018). Chemical characterization, antioxidant properties and oxygen consumption rate of 36 commercial oenological tannins in a model wine solution. *Food Chemistry*, 268, 210–219. https://doi.org/10.1016/j.foodchem.2018.06.031

Walker, R.R., Read, P.E. & Blackmore, D.H. (2000). Rootstock and salinity effects on rates of berry maturation, ion accumulation and colour development in Shiraz grapes. *Australian Journal of Grape and Wine Research*, 6, 227-239.

Walker, R.R., Blackmore, D.H., Clingeleffer, P.R. & Correll, R.L. (2002). Rootstock effect on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana). Yield and vigour inter-relationships. *Australian Journal of Grape and Wine Research*, 8, 3–14. https://doi.org/10.1111/j.1755-0238.2002.tb00206.x

Walker, R.R., Deidre, H., Blackmore, R., Ray, L. & Correll, A. (2004). Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana). Ion concentrations in leaves and juice. *Australian Journal of Grape and Wine research*, 10, 90-99. https://doi.org/10.1111/j.1755-0238.2004.tb00011.x

Walker, R.R., Blackmore, D.H. & Clingeleffer, P.R. (2010). Impact of rootstock on yield and ion concentrations in petioles, juice and wine of Shiraz and Chardonnay in different viticultural environments with different irrigation water salinity. *Australian Journal of Grape and Wine Research*, 16, 243-257. https://doi.org/10.1111/j.1755-0238.2009.00081.x.