Effects of Water Stress on the Phenolic Compounds of ‘Merlot’ Grapes in a Semi-Arid Mediterranean Climate

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Abstract: Of all the abiotic stress types to which plants grown in fields are exposed, the most influential is water stress. It is well accepted that adopting controlled deficit irrigation strategies during the growing season has beneficial effects on the chemical compositions of grapes and red wines. However, there is a discrepancy in the timing, intensity and duration of deficit. This study aimed to evaluate the changes in phenolic composition of ‘merlot’ cultivar grapes when subjected to different levels of water stress in a semi-arid Mediterranean climate. Four treatments with different water stress levels were applied within two phenological intervals (flowering-veraison, veraison-maturity) to 128 grapevines for two consecutive years. The water stress levels for Treatments 1, 2, 3 and 4 were: no-light, light-moderate, moderate-intense and intense for the flowering-veraison and veraison-maturity intervals, respectively. Water stress distinctly affected the phenolic compounds in skin and seeds. The concentrations of flavan-3-ols and total polyphenols were much higher in seeds than in skin, and in both fractions, tannins are the major compounds.

Keywords: abiotic stress; cultivar; flavonoids; grape; water deficit

1. Introduction

Of all the abiotic stress types to which plants grown in fields are exposed (drought, salinity, soil acidification, high temperature, high radiation), the most influential is water stress. In the electron transport chain of the light phase of photosynthesis, water is the first electron donor, and therefore, its participation is essential for plants to carry out this process. It also participates in biochemical reactions, and transports synthesized materials and products to ensure many metabolic functions. Finally, through evaporation, it protects organisms against warming [1].

The vine is one of the most abundant and important crops on our planet [2]. Its wide and deep root system, its efficient mechanism of stomatal control and its capacity to perform osmotic adjustment [3–5] allow it to adapt well to cultivation in semi-arid or arid climates. Thus, it is considered a water deficit-tolerant species [6,7]. The response of vine to water stress depends on its variety and ability to adapt to the environment [8–13]. It consists mainly in lowering transpiration and photosynthesis rates [8,14–21] to prevent physiology from being severely affected. One of the most widely used methodologies to ascertain the vine water status is determining the leaf water potential ($\Psi_w$) [22–26]. When a plant and soil are in equilibrium, the predawn leaf water potential ($\Psi_{PD}$) measurement provides information about the matrix potential of soil in the root zone and well reflections a plant’s general water status.
From the enological point of view, higher quality production in grapevine is normally achieved under non-optimal growing conditions (stressful conditions), hence the water deficit objective is to guarantee high fruit quality and to improve sustainable water use [27]. A consensus has been reached by viticultural researchers about adopting controlled deficit irrigation strategies during the growing season because this has beneficial effects on the chemical composition of grapes and red wines [28–31]. However, there is a discrepancy in the timing, intensity and duration of deficit. This is because not all of varieties have the same sensitivity to stress in different phenological states [32].

Both the expression of genes and, the activation of the enzymes involved in the biosynthesis of phenolic compounds depend fundamentally on three factors: climate conditions, phenological state and cultural practices. Among cultural practices, water status management is considered one of the most important tools to increase polyphenols content and to improve the quality of red grapes [7,20,33–37]. It is generally considered that deficit irrigation regimes stimulate synthesis and favor the concentration of phenolic compounds in grapes [37–40].

La Mancha Designation of Origin (DO) is one of the largest wine growing areas in the world, where ‘merlot’ is an authorized cultivar. This area has a semi-arid climate, and currently, water resources for vine are limited by law to 1350 m$^{3}$/ha and year. However, in the near future, and because of climate change, both an increased water deficit and decreased water reserve are expected in this area. Therefore, it is not foreseeable that a greater water deficit can be supplied by a higher irrigation dose. The aim of the current study was to evaluate the changes on the phenolic compounds in the ‘merlot’ cultivar grapes when subjected to different water stress levels, maintained by irrigation management, for two consecutive growing seasons. This study also provides some insight into the suitability of ‘merlot’ cultivar in this DO for the coming years.

2. Materials and Methods

2.1. Study Area

The trial was carried out over two years in a vineyard located in the municipality of Argamasilla de Alba (La Mancha DO). The geographical coordinates of the area are: 39°08′10″ North; 3°04′00″ West and its altitude is 670 m.a.s.l. According to the Winkler Index, this area is classified as Region IV, with scarce rainfall all year long (about 350 mm), with less than 50% occurring during the grapevine growing season. The reference evapotranspiration value (ET$_0$) is about 1300 mm/year, which exceeds 1000 mm during the active vegetation period. Both study years were exceptionally dry, with only 29.8 and 61.9 mm of rainfall in spring the Study Year 1 and Study Year 2, respectively. ET$_0$ values of 1087 and 1056 mm for Study Year 1 and Study Year 2, respectively were recorded between April 1 and September 30 (Figure 1).

The soil type on which the vineyard lies is one of the most representative in the La Mancha region, Petric Calcisols [41] and Petrocalcic Calcixerept [42], showing powerful petro-calcic horizons and limited thickness, sometimes less than 40 cm (Figure 2). The main characteristic of this soil is the presence of a C$_{km}$ horizon, who thickness is sometimes more than 1 m. It is practically impenetrable to grapevine roots and thus, conditions plant cultivation. Pedoclimatic soil conditions correspond to a xeric moisture regime that is typical of Mediterranean climates.
2.2. Plant Material

The study was carried out in a vineyard planted with the variety ‘merlot’ (*Vitis vinifera* L.) grafted onto Fercal rootstocks (5 years old). The main characteristic of this rootstock is its high resistance to chlorosis [43] and its adaptation to calcareous soils. It resists up to 60% total limestone, 40% active limestone and a chlorinating power index (CPI) of 120. Plants were arranged in rows set 3 m apart and the spacing between grapevines was 1.2 m. The planting density was 2778 grapevines/ha, which was conducted on a 3-wire trellis in double Royat cord. Each arm had 3–4 shoots pruned to two buds. Row orientation was 340° N-NW/160° S-SE. In order to adjust the number of both shoots (12 per plant) and
bunches (24–26 per plant) in all the grapevines, shoots were removed from arms to obtain an equal number in all grapevines every year before flowering.

2.3. Experimental Design

Four experimental treatments were designed. In each case, grapevines were subjected to different water restriction levels within two phenological intervals (flowering-veraison, veraison-maturity) by leaving their $\Psi_{PD}$ within certain ranges, which were adapted from those established by Carbonneau [22] (see Table 1). Treatments were applied to 128 grapevines, and distributed into two blocks of 64 grapevines each. Four treatments were distributed randomly to each block. In Year 1, each treatment involved 16 consecutive plants located in the same row. However, in Year 2, a decision was made to improve the representativeness of the mean values in relation to grape phenolic compounds. For this reason, each treatment was divided into two subtreatments and, consequently, the number of samples (n) rose from two to four. Each subtreatment consisted of eight consecutive vines of the 16 making up the treatment.

Table 1. Predawn water potential ($\Psi_{PD}$) ranges and stress levels supported by grapevines in different treatments, adapted from Carbonneau.

| Treatment | Period | Vine Water Status | Type of Stress |
|-----------|--------|------------------|---------------|
| $T_{1}(-0.2;−0.2)$ | $0 \text{ MPa} \geq \Psi_{PD} \geq −0.2 \text{ MPa}$ | $\Psi_{PD} \geq −0.2 \text{ MPa}$ | No-Light |
| $T_{2}(-0.2–0.4;−0.4)$ | $−0.2 \text{ MPa} > \Psi_{PD} \geq −0.4 \text{ MPa}$ | $\Psi_{PD} \geq −0.4 \text{ MPa}$ | Light-Moderate |
| $T_{3}(-0.4–0.6;−0.6)$ | $−0.4 \text{ MPa} > \Psi_{PD} \geq −0.6 \text{ MPa}$ | $\Psi_{PD} \geq −0.6 \text{ MPa}$ | Moderate-Intense |
| $T_{4}(-0.6;−0.8)$ | $−0.6 \text{ MPa} > \Psi_{PD}$ | $\Psi_{PD} \geq −0.8 \text{ MPa}$ | Intense |

2.4. Water Regime

In order to keep plants within the chosen ranges for each treatment, an irrigation calendar was established to provide different amounts of water. The amount of water varied depending on the treatment itself, weather condition, and cycle time (see Table 2). The employed irrigation system was drip and lines were suspended from the forming wire. Drippers operated at a flow rate of 2.2 L/h and were set 0.75 m apart. Watering was done at night to achieve maximum effectiveness and to avoid losses.

Table 2. Irrigation period and volumes of water in the different treatments during the two-year trial.

| Treatment | Year | Repetition | Volume (mm) |
|-----------|------|------------|-------------|
|           |      |            | Total | Average |
| $T_{1}(-0.2;−0.2)$ | 1 | T1-1 | 131.01 | 132.27 |
|            |    | T1-2 | 133.54 |     |
|            | 2 | T1-1 | 132.69 | 131.71 |
|            |    | T1-2 | 130.72 |     |
| $T_{2}(-0.2–0.4;−0.4)$ | 1 | T2-1 | 114.95 | 117.04 |
|           |    | T2-2 | 119.14 |     |
|           | 2 | T2-1 | 110.37 | 110.12 |
|           |    | T2-2 | 109.86 |     |
Table 2. Cont.

| Treatment | Year | Repetition | Volume (mm) |
|-----------|------|------------|-------------|
|           |      |            | Total       | Average     |
| T3(−0.4–0.6;−0.6) | 1    | T3-1       | 93.82       | 93.07       |
|           |      | T3-2       | 92.33       |             |
|           | 2    | T3-1       | 66.12       | 67.36       |
|           |      | T3-2       | 68.61       |             |
| T4(−0.6;−0.8)   | 1    | T4-1       | 70.95       | 70.63       |
|           |      | T4-2       | 70.31       |             |
|           | 2    | T4-1       | 55.22       | 56.46       |
|           |      | T4-2       | 57.70       |             |

2.5. Water Potential

Grapevine water status was evaluated by measuring $\Psi_{PD}$ [44]. Measurements were taken between the flowering and maturity phenological stages with a Scholander pressure chamber (SKPM-1400, Skye Inst. Lim., UK). Measurements were taken on Days 1, 3 and 5 of each week for 33 days in Year 1 and for 34 days in Year 2. Every year, two series of three and five consecutive days were carried out to more accurately track $\Psi_{PD}$ behavior. Each daily $\Psi_{PD}$ datum assigned to every treatment corresponded to the average of eight measurements taken of eight leaves from eight different grapevines.

2.6. Grape Phenolic Composition

In order to determine the parameters related to grape phenolic composition, duplicate samples of 100 grapes were used per repetition. The result for each treatment was calculated as the mean of the mean values of all the repetitions (two samples from Year 1 and four from Year 2).

2.6.1. Extracting the Phenolic Fraction of Skin and Seeds

For each treatment and repetition, 100 healthy berries were weighed and then carefully peeled to remove pulp. The obtained seeds and skin were washed 3 times in water (Milli-Q of 18 mΩ) and gently dried twice by placing them between sheets of filter paper before being weighed. Subsamples of skin (16 g) and seeds (2 g) were extracted twice with 100 mL of the 50:48.5:1.5 (v/v/v) CH$_3$OH/H$_2$O/HCOOH mixture [45] by a homogenizer (Heidolph DIAx 900) for 2 min to be subsequently centrifuged at 2500 rpm for 15 min. The supernatants from the two extractions were put together and stored at $-20 ^\circ$C until analyzed.

2.6.2. Analyzing Phenolic Compounds

Conventional methods were followed to analyze phenolic compounds. Anthocyanin concentrations were determined by the sulfur discoloration method [46], and catechins according to the reaction method with dimethylaminocinnamaldehyde (DMACH), and they were measured at 640 nm [47]. Tannin was established by the acid hydrolysis method, and was catalyzed by ferric sulfate with subsequent stabilization with 1-butanol and was measured at 550 nm [48]. Total polyphenols were determined by measuring absorbance at 280 nm after appropriate sample dilution [49].

2.7. Statistical Analysis

The statistical analysis of the results was performed with version 23.0 of the SPSS statistical package. The possible effect of treatments on the different considered variables considered was evaluated by performing an analysis of variance. Means were compared by Tukey’s honestly significance difference test, with a probability level of $p \leq 0.05$ (*).
The comparison between the means of the year was assessed by a Student’s t-test for independent samples, with a probability level of $p \leq 0.05$ (*).

3. Results

3.1. Grape Composition

Grape weight for Year 2 decreased in relation to Year 1 for all treatments (17.4%, 20.2%, 28.3% and 29.2% for T1, T2, T3 and T4, respectively) (Table 3). In Year 1, the reductions in grape in T2, T3 and T4 vs. T1 were 4.6%, 9.2% and 12%, respectively, which were considerably more marked in Year 2 (7.8%, 21.1% and 24.4% for T2, T3 and T4, respectively). Between both years, significant differences were observed (0.78 ± 0.10 a and 1.02 ± 0.06 b for Years 2 and 1, respectively) and the grape weight lowered by 23.5% in Year 2 compared to Year 1.

Table 3. Means and standard deviations of grape weight and the different fractions making it up: grape weight (W, g), weight percentage of skin/grape weight (% Skin/W, %), weight percentage of seeds/grape weight (% Seeds/W, %), weight percentage of pulp/grape weight (% Pulp/W, %) for all the four water regime treatments in the 2 years.

| Year | Treatment | Samples | $W^z$ | % Skins/W | % Seeds/W | % Pulp/W |
|------|-----------|---------|-------|-----------|-----------|----------|
|      | ($\Psi_{FP}$) | (n) | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 1    | $T_1(-0.2; -0.2)$ | 2 | 1.09 ± 0.02 | 17.96 ± 0.16 | 5.76 ± 0.25 a | 76.28 ± 0.10 |
|      | $T_2(-0.2; -0.4)$ | 2 | 1.04 ± 0.07 | 15.91 ± 2.14 | 6.14 ± 0.01 ab | 77.95 ± 2.16 |
|      | $T_3(-0.4; -0.6)$ | 2 | 0.99 ± 0.08 | 16.41 ± 0.08 | 6.15 ± 0.27 ab | 77.44 ± 0.35 |
|      | $T_4(-0.6; -0.8)$ | 2 | 0.96 ± 0.07 | 14.73 ± 1.41 | 6.73 ± 0.01 b | 78.54 ± 1.41 |

**Sig**: ns

| Year | $T_1(-0.2; -0.2)$ | 4 | 0.90 ± 0.03 c | 14.12 ± 2.63 | 4.53 ± 0.07 a | 81.34 ± 2.65 |
|      | $T_2(-0.2; -0.4)$ | 4 | 0.83 ± 0.04 b | 13.99 ± 2.30 | 5.31 ± 0.12 b | 80.70 ± 2.35 |
|      | $T_3(-0.4; -0.6)$ | 4 | 0.71 ± 0.03 a | 13.53 ± 1.15 | 5.76 ± 0.28 c | 80.70 ± 1.28 |
|      | $T_4(-0.6; -0.8)$ | 4 | 0.68 ± 0.03 a | 14.57 ± 1.06 | 6.11 ± 0.28 c | 79.31 ± 1.25 |

| Year 1 | 8 | 1.02 ± 0.06 b | 16.25 ± 1.58 | 6.19 ± 0.40 | 77.55 ± 1.30 a |
| Year 2 | 16 | 0.78 ± 0.10 a | 14.05 ± 1.94 | 5.40 ± 0.60 | 80.51 ± 1.94 b |

* Number of samples (n) for grape weight is n = 8 in Year 1 and n = 16 in Year 2. y The letters indicate statistically significant differences between treatments according to Tukey’s honestly significant difference test ($\alpha = 0.05$). A comparison was made between the means of years by Student’s t-test for independent samples ($\alpha = 0.05$). ** Significance (Sig): *, **, ***: ns, significant at $p \leq 0.05, 0.01, 0.001$, or not significant, respectively.

For skin, no significant differences between treatments or years were found. The average percentages of Year 2 lowered compared to Year 1 for all treatments (21.4%, 12.1%, 17.5% and 1.1% for T1, T2, T3 and T4, respectively). During Year 1, the skin weight percentage lowered in relation to T1 in other treatments (11.4%, 8.6% and 18% for T2, T3 and T4, respectively). However, in Year 2, this percentage decreased only for T2 and T3 (0.9% and 0.2% for T1, T2, T3 and T4, respectively). When treatments were compared between both years, the trend was the same as for skin. The average percentage dropped in relation to Year 1 for all years by Student’s t-test for independent samples ($\alpha = 0.05$). A comparison was made between the means of years by Student’s t-test for independent samples ($\alpha = 0.05$). For seeds, significant differences between treatments were observed within the same year (5.76 ± 0.25 a, 6.14 ± 0.01 ab, 6.15 ± 0.27 ab and 6.73 ± 0.01 b for T1, T2, T3 and T4, respectively, in Year 1 and 4.53 ± 0.07 a, 5.31 ± 0.12 b, 5.76 ± 0.28 c and 6.11 ± 0.28 c for T1, T2, T3 and T4, respectively, in Year 2). However, no significant differences appeared between different years. In seeds, the increasing water stress trend was the opposite to that of skin when treatments were compared within the same year. During Year 1, the increased grape seed weight percentage in T2, T3 and T4 vs. T1 was 6.6%, 6.8% and 16.8%, respectively, while these increases were much greater in Year 2 (17.2%, 27.2% and 34.9% for T2, T3 and T4, respectively). When treatments were compared between both years, the trend was the same as for skin. The average percentage dropped in relation to Year 1 for all years by Student’s t-test for independent samples ($\alpha = 0.05$). A comparison was made between the means of years by Student’s t-test for independent samples ($\alpha = 0.05$).
treatments (21.3%, 13.5%, 6.3% and 9.2% for T1, T2, T3 and T4, respectively). Between both study years, the grape seed weight percentage also dropped by 12.8% in Year 2 compared to Year 1.

Unlike the other fractions, the pulp weight percentage values were higher in Year 2 for all treatments (6.6%, 3.5%, 4.2% and 0.1% for T1, T2, T3 and T4, respectively). The pulp weight percentage increased in Year 1 vs. T1 for the other treatments (2.2%, 1.5% and 3% for T2, T3 and T4, respectively), with a decreasing trend in Year 2 (0.8% for T2, T3 and 2.5% for T4). Significant differences between years appeared (77.55 ± 1.30 a and 80.51 ± 1.30 b for Years 1 and 2, respectively) and the weight percentage increased by 3.8% in Year 2 vs. Year 1.

### 3.2. Phenolic Compounds in Skin

The differences in stress levels maintained in the different treatments slightly affected the skin phenolic components (Table 4). Significant differences appeared in monomeric flavan-3-ols compounds—catechins—only in Year 2 (98.5 ± 7.4 a, 122 ± 19 b, 96.2 ± 6.5 a and 104.2 ± 6.4 ab for T1, T2, T3 and T4, respectively), and there was no evidence that the water stress level caused them. However, the differences in concentrations between both years were obvious for all the phenolic compounds, but significant differences were observed only in total polyphenols (631 ± 65 a and 1217 ± 125 b for Years 2 and 1, respectively). The concentrations of anthocyanins, catechins, tannins and total polyphenols decreased (19.7%, 48.9%, 9.9% and 48.2%, respectively) in Year 2 vs. Year 1.

### Table 4. Means and standard deviations of skin phenolic compounds: anthocyanins (Ant, mg of malvidin/kg grape), catechins (Cat, mg of catechin/kg grape), tannins (Tan, g of tannin/kg grape) and total polyphenols (T.P., mg of gallic acid/kg grape) for all the four water regime treatments in both years.

| Year | Treatment | Samples | Ant | Cat | Tan | T.P. |
|------|-----------|---------|-----|-----|-----|------|
|      |            | (n)     | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 1    | T1(0.2−−0.2) | 2       | 1017 ± 32 | 184.6 ± 15.2 | 1.87 ± 0.09 | 1130 ± 34 |
|      | T2(−0.2−−0.4) | 2       | 1194 ± 152 | 230.1 ± 2.6 | 2.19 ± 0.26 | 1203 ± 171 |
|      | T3(−0.4−−0.6) | 2       | 1111 ± 157 | 206.1 ± 36.9 | 2.00 ± 0.22 | 1177 ± 143 |
|      | T4(−0.6−−0.8) | 2       | 1071 ± 156 | 201.8 ± 4.6 | 2.01 ± 0.13 | 1357 ± 27 |
|      | Sig        | ns      | ns     | ns   | ns   |
| 2    | T1(0.2−−0.2) | 4       | 816 ± 65 | 98.5 ± 7.4 a | 1.82 ± 0.22 | 628 ± 63 |
|      | T2(−0.2−−0.4) | 4       | 930 ± 156 | 122 ± 19 b | 1.76 ± 0.29 | 651 ± 100 |
|      | T3(−0.4−−0.6) | 4       | 882 ± 42 | 96.2 ± 6.5 a | 1.77 ± 0.24 | 615 ± 26 |
|      | T4(−0.6−−0.8) | 4       | 900 ± 83 | 104.2 ± 6.4 ab | 1.93 ± 0.14 | 631 ± 73 |
|      | Sig        | ns      | *      | ns   | ns   |
|      | Year 1     | 8       | 1098 ± 123 | 205.7 ± 23.1 | 2.02 ± 0.19 | 1217 ± 125 b |
|      | Year 2     | 16      | 882 ± 97 | 105.2 ± 14.4 | 1.82 ± 0.22 | 631 ± 65 a |

* Letters indicate statistically significant differences between treatments, according to Tukey’s honestly significant difference test (α = 0.05). A comparison made between the means of years was performed by the Student’s t-test for independent samples (α = 0.05). ** Significance (Sig): *, **, ***: ns: significant at p ≤ 0.05, 0.01, 0.001, or not significant, respectively.

### 3.3. Phenolic Compounds in Seeds

The seed phenolic components, unlike those of skin, were affected more by the water stress level (Table 5). A slight upward trend was found in all the phenolic compounds as the water stress level rose. Significant differences between treatments were observed for Year 2 for catechins (855 ± 279 a, 1373 ± 98 b, 1397 ± 101 b and 1348 ± 107 b for T1, T2, T3 and T4, respectively), tannins (4.03 ± 0.97 a, 6.25 ± 0.73 ab, 6.82 ± 1.67 b and 6.51 ± 0.79 b for T1, T2, T3 and T4, respectively) and total polyphenols (1298 ± 426 a, 1796 ± 29 ab, 1959 ± 178 b and 1923 ± 172 b for T1, T2, T3 and T4, respectively), and for catechins between the years (1742 ± 354 a and 2403 ± 362 b for Years 2 and 1, respectively).
Table 5. Means and standard deviations of seed phenolic compounds: catechins (Cat, mg of catechin/kg grape), tannins (Tan, g of tannin/kg grape) and total polyphenols (T.P., mg of gallic acid/kg grape) for all the four water regime treatments in both years.

| Year | Treatment (ΨPD) | Samples | Cat Mean ± SD | Tan Mean ± SD | T.P. Mean ± SD |
|------|-----------------|---------|---------------|---------------|---------------|
| 1    | T1 (0.2; −0.2)  | 2       | 1279 ± 38     | 4.91 ± 0.10   | 2083 ± 91     |
|      | T2 (−0.2; −0.4) | 2       | 1333 ± 39     | 5.30 ± 0.56   | 2290 ± 123    |
|      | T3 (−0.4; −0.6) | 2       | 1372 ± 44     | 5.60 ± 0.19   | 2325 ± 13     |
|      | T4 (−0.6; −0.8) | 2       | 1442 ± 86     | 5.45 ± 1.20   | 2564 ± 250    |
| Sig  |                 |         | ns            | ns            | ns            |
| 2    | T1 (0.2; −0.2)  | 4       | 855 ± 279     | 4.03 ± 0.97   | 1298 ± 426    |
|      | T2 (−0.2; −0.4) | 4       | 1373 ± 98     | 6.25 ± 0.73   | 1796 ± 29     |
|      | T3 (−0.4; −0.6) | 4       | 1397 ± 101    | 6.82 ± 1.67   | 1959 ± 178    |
|      | T4 (−0.6; −0.8) | 4       | 1348 ± 107    | 6.51 ± 0.79   | 1923 ± 172    |
| Sig  |                 |         | ***           | *             | **            |
|      | Year 1          | 8       | 1369 ± 75     | 6.17 ± 1.66   | 2403 ± 362    |
|      | Year 2          | 16      | 1243 ± 275    | 5.90 ± 1.50   | 1742 ± 354    |

*Letters indicate statistically significant differences between treatments, according to Tukey’s honestly significant difference test (α = 0.05). A comparison made between the means of years was performed by the Student’s t-test for independent samples (α = 0.05). * Significance (Sig): *, **, ***: significant at p ≤ 0.05, 0.01, 0.001, or not significant, respectively.

Except for T1, the catechin concentrations were similar to the other treatments in both years and were similar between treatments within the same year. However, differences for tannins and total polyphenols were recorded.

In tannins, the average concentrations obtained in Year 2 rose compared to Year 1 for treatments T2, T3 and T4 (18%, 21.8% and 19.4%, respectively), while they lowered 17.9% in T1. In Year 1, the increases in the grape seed weight percentage in T2, T3 and T4 compared to T1 were 7.9%, 14.1% and 11%, respectively, and these increases were much greater in Year 2 (55.1%, 69.2% and 61.6% for T2, T3 and T4, respectively).

In total polyphenols, the trend in relation to an increasing water stress was similar to that of tannins when treatments were compared within the same year, but the opposite occurred when comparing both years. In Year 1, the increases in the total polyphenol concentrations in T2, T3 and T4 vs. T1 were 9.9%, 11.6% and 23.1%, respectively, but these increases were much greater in Year 2 (39.3%, 52% and 49.2% for T2, T3 and T4, respectively). However, when treatments were compared between both years, the concentrations of Year 2 were lower than those in Year 1 (38.1%, 21.6%, 15.7% and 25% for T1, T2, T3 and T4, respectively).

Between years, the concentrations of all the phenolic compounds lowered in Year 2 compared to Year 1 (9.2%, 4.4% and 27.5% for catechins, tannins and total polyphenols, respectively).

4. Discussion

4.1. Grape Composition

According to the results herein obtained, both grape weight and that of the different fractions making it up were affected by water stress. However, the effects and their intensity differed depending on the fraction. In this study, as a rule, the weight percentage of seeds and pulp in grape tends to increase with stress severity, while grape weight and the skin weight percentage tend to decrease, but this is less evident in the latter case. These findings partly agree with those obtained by other authors in Australia when evaluating the ‘syrah’ cultivar in two different water regimes (deficient and irrigated). Those authors observed how the grapes from stressed grapevines were smaller in size and had a higher proportion...
of seeds per weight. In contrast, they noted that grapes had a higher proportion of skin and a lower proportion of pulp [50]. These differences could be due to the area where trials were carried out as vine and cultivar management may differ. In previous studies performed under different water supply conditions, pulp was the most determining factor for grape weight, and a positive correlation between seed weight and pulp weight was established [37]. Like our results, these cited studies did not observe any significant differences in the skin weight percentage compared to that of berry, but also recorded a slight downward trend in this ratio with increasing water stress. These authors also did not appreciate significant in the weight percentage of the skins with respect to that of the berry, but they observed a slight downward trend in this relationship as the level of water stress increased in the treatments. Similar conclusions have been reached by other authors when comparing ‘cabernet sauvignon’ cultivar behavior in different water regimes as berries were smaller in size in those that were water-deficient. This is in terms of the reduction that occurs in the mesocarp (pulp), rather than to what happens to the exocarp (skin) or seed weight [51]. It has been proved that the impact of both seed weight and number on total grape weight is due to the hormonal regulation that seeds exert on cell proliferation and expansion [52–54].

The relations between different berry components provide information about the enological potential of grape [54,55]. The seed weight/grape weight ratio affects the time when grapes begin to ripen and, therefore, the accumulation rate of phenolic compounds. In grapes, the seed weight/berry weight ratio also affects when the grapes begin to ripen, and therefore, the rate of accumulation of phenolic compounds. This ratio is higher begin to ripen later than those in which it is low [56]. Previous reports on the relation between berry size and the qualitative characteristics in the ‘syrah’ cultivar have revealed that the skin weight/berry weight ratio does not change when berry size grows, whereas the seed weight/berry weight ratio increases [55]. These findings contrast with those herein obtained because the skin weight/berry weight ratio slightly rose when berry size increased, while the seed weight/berry weight ratio lowered. These differences may be due to the fact that all the grapes from each treatment were herein considered regardless of their size, and were not previously grouped into categories, or because they did not come from the same variety.

4.2. Phenolic Compounds in Skin and Seeds

Grape quality is largely defined by the concentration of the different phenolic compounds that it contains, which are not evenly distributed throughout grape. Skin contains mainly anthocyanins, flavonols, hydroxycinnamic acids, monomeric flavan-3-ols and proanthocyanidins, while monomeric flavan-3-ols and proanthocyanidins predominate in seeds, and hydroxycinnamic acids do so in pulp [57]. The biosynthesis of phenolic compounds is influenced by temperature, particularly by UV radiation. Flavonols and anthocyanins increase in UV-exposed grape skin, whereas flavonols and hydroxybenzoic acids show no significant change [58]. In this study, he analyzed compounds were flavonoids (anthocyanins and flavan-3-ols) because, apart from constituting a significant percentage of the material phenolic present in grapes, they are the most important group from the enological point of view. These compounds are essential for wine, especially red wine, for being involved in some of their main characteristics, such as structure, astringency, bitterness, color and antioxidant power [59–61]. Others properties of these compounds are also able to influence the properties of some volatile compounds [62] by either enhancing their aroma or masking it [63], and they have beneficial effects on the organism because they act as cardiac protectors, antioxidants and anticancer agents [64–67].

Strong correlations have been found among grape weight, anthocyanin concentration and skin color parameters [51]. According to a classic viticulture postulate, the higher skin-to-flesh ratio of smaller berries leads to grapes possessing higher phenolic substances [55,68]. Conversely in assays performed under water deficit conditions, the concentration of phenolic compounds in grapes is not only due to berry size, but also to changes
in vine metabolism triggered by factors like the water status, cultural practices or weather conditions that occur during the season \[29,69–71\]. In our study, water stress slightly affected skin phenolic compounds, and the differences in their concentrations were bigger between years. These results agree with those obtained by other authors \[34,69,72–75\], who considered that an altered vine water status would affect the biosynthesis of flavonoids in skin only slightly, and its impact would not depend that much on the plant water status, but on the year \[51,55,76\]. In contrast, other studies have reported significantly higher concentrations of anthocyanins and tannins in grapes from stressed grapevines than in those obtained from non-stressed ‘cabernet sauvignon’ \[77\], ‘tempranillo’ \[38\], and ‘merlot’ cultivars \[78\] in the same year. It is generally considered that anthocyanin synthesis is promoted when water stress occurs at the end of the growing season, between the veraison and harvest stages \[79\]. The impact of water stress on anthocyanin accumulation in grapes is cultivar-dependent \[80\].

In both our study years, the amount of anthocyanins improved with increasing berry weight when expressing their concentrations as mg/berry. Total polyphenols decreased with increasing berry weight when expressing values as both mg/kg of grape and mg/g of skin. These findings are consistent with those obtained by other researchers, who have also reported varying results depending on the units in which concentrations of total anthocyanins were expressed (mg/berry, mg/g skin, mg/kg grape and mg/cm² skin) \[55\]. In line with other reports, no significant differences in seed weight were found between treatments \[81\]. However, the reduced berry weight led to a greater contribution of seeds, expressed as a percentage, to the total grape weight. Due to the close relation between berry seeds and berry size, it has been suggested that berry size might influence not only the skin/pulp ratio, but also the seed/pulp ratio \[38,55,82\]. As grape seeds contain monomeric flavan-3-ols and proanthocyanidins, the seed/pulp ratio could also strongly affect grape phenolic composition.

All treatments, regardless of their intensity, increased the phenolic content in grapes. The choice of stress levels will depend on the type of wine you want to make. If the water stress level is intense, the grapes will have a higher concentration of phenols but the yield will be drastically lowered, mainly due to the decrease in the size of the berry \[83\]. Conversely, stress levels in a range between light and moderate, allow significantly improving the quality of the berry and maintaining the yield.

5. Conclusions

In this study, water stress affected skin and seeds differently. The concentrations of anthocyanins, catechins, tannins and total polyphenols in skin scarcely varied, while an increase in the concentration of all the phenolic compounds took place in seeds at higher stress levels. The concentrations of flavan-3-ols and total polyphenols were much higher in seeds than in skin, and tannins were major compounds in both fractions.

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