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Effects of water stress on the sugar accumulation and organic acid changes in Cabernet Sauvignon grape berries

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Abstract: The eastern foot of Helan Mountains in Ningxia belongs to the semiarid area and has been identified as the best wine grape producing area in China. In order to solve the problems of a high sugar content, organic acid falling too fast and severe shortage of acidity in the berries during ripening, which lead to lack of wine harmony, this study took *Vitis. vinifera* L. cv. 'Cabernet Sauvignon' as the experimental material and expanded nine treatments from setting to harvest. Nine rows were selected and divided into three groups, treated with mild (–0.20 MPa ≥ Ψ_b ≥ –0.40 MPa), moderate (–0.40 MPa ≥ Ψ_b ≥ –0.60 MPa), severe (Ψ_b ≥ –0.60 MPa) water stress from setting to veraison separately. From veraison to harvest, within each group, the mild, moderate, severe water stress were set, forming light-light (CK), light-medium (T1), light-heavy (T2), medium-light (T3), medium-medium (T4), medium-heavy (T5), heavy-light (T6), heavy-medium (T7), heavy-heavy (T8) treatments. The results showed that when the medium treatment was used from setting to veraison and the medium or severe treatment was used during post-veraison to harvest, it would facilitate the transportation and accumulation of sugar and improve the fruit quality.

Key words: water treatment; sugar transportation; sugar metabolism; organic acid synthesis

Grapevines (*Vitis vinifera* L.) have had deep ties to human culture for more than 5 000 years (McGovern et al. 1996). Being the most widely cultivated and economically important fruit crop in the world, this crop plays a key role in many countries' economies, with a global market size of over 31.4 billion euros (OIV 2019). Sugar transport and partitioning are important for normal plant growth (Julius et al. 2017). Grape berries are typical non-transitional fruits, and their growth shows a typical double-S curve, which could be divided into three periods. The first and third stages are rapid growth periods, and the second stage is a stagnate phase (Fillion et al. 1999). Sugar is transported in the form of sucrose from the source organ (leaf) through the phloem to the vascular phloem of the fruit. Sugar arrives at the sieve element-companion cell (SE-CC) complex in the fruit phloem and enters the sink cell through two cytological pathways: one is the symplast pathway, that is, the sugar is transported to the sink cell through the intercellular filament between the sieve element companion cell complex and the surrounding phloem parenchyma cells; the other is the apoplast pathway, that is, sugar is unloaded from the sieve-associated cell complex to the ectoplasmic space, and then enters the cell through the carrier or proton pump (H^+–ATPase) on the plasma membrane of the cell. At the same

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time, the unloading pathways of grapes in different stages are also different, and the two pathways are inter-convertible, that is, from the symplast pathway to the apoplast pathway (Lalonde et al. 2003). Zhang et al. (2006) found that in stage I and II of the grape fruit development, the unloading mode of the vascular bundle in the phloem of the fruits is mainly through the apoplast unloading, while in stage III of the fruit development, the apoplast pathway was blocked, and the unloading mode is transformed into apoplast unloading.

Sugar accumulation is an important event in the physiological process of ripening grapes, and is an important feature of a grape's excellent physiological characteristics and product value (Kambiranda et al. 2011). Sugar metabolism is not only essential to this process, but the sugar also functions as a kind of signal molecule (Rolland et al. 2006). Synthesised carbohydrates must be transported from the leaves (source tissues) to the non-photosynthetic organs (sink tissues) such as the roots and seeds (Chenu, Scholes 2015). Sugar utilisation is, therefore, a key factor that promotes plant growth and development (Yue et al. 2015). The process of sugar accumulation in a fruit is regulated by many factors. As a powerful metabolic sink, fruit obtains a large amount of assimilates from source tissues during the maturation process. The ability of the fruit to obtain assimilates is largely determined by the strength of the sink, while the strength of the sink is determined by the concentration of the sucrose in the fruit (Lemoine et al. 2013). As an osmotic substance, signalling the molecules and nutrients, sucrose plays a variety of important roles in tolerance to stress (Gupta, Kaur 2005; Ruan et al. 2010; Gong et al. 2015). Therefore, the strength of the reservoir is determined by controlling the enzymes related to the sucrose synthesis, thus regulating the synthesis of the assimilates (Moriguchi et al. 1990, Hubbard et al. 1991). Invertase, sucrose synthase (SS) and sucrose phosphate synthase (SPS) regulate the sugar accumulation and metabolism in grape berries (Copeland 1990). Sucrose synthase (SS) is a highly regulated cytoplasmic enzyme that catalyses the conversion of sucrose and nucleoside diphosphate into nucleoside diphosphate glucose and fructose (Li et al. 2013). Invertase can be divided into acid invertase (AI) and neutral invertase (NI) according to the pH (Avigad 1982).

In recent years, studies on deficit irrigation have been further deepened, and it has been pointed out that the grapes have a higher skin to flesh ratio when water is in short supply, so it can increase the concentration of major solutes related to a wine's quality (Triolo et al. 2019). Water stress significantly increases the content of non-acylated anthocyanins in wine (Liang et al. 2011). Weak light and water stress contribute to the improvement of grape peel and wine phenols, and to the reduction of methoxypyrazines in the wine while achieving high yields (Brillante et al. 2018). A water deficit affects the concentration of the total phenols, total anthocyanins and chromaticity (Cáceres-Mella et al. 2018). The use of partial root zone drought irrigation could improve the water interception efficiency, therefore affecting the berry quality (Ezzahouani et al. 2007). Regulated deficit irrigation (RDI) significantly reduces the titratable acidity and increases the pH of the total soluble solids (Ju et al. 2019). Previous studies suggested that short-term water stress can improve the fruit soluble solids content and can reduce the total acidity and increase the resveratrol content in fruits (Herrera et al. 2017). Water stress can regulate the accumulation of green leaf volatiles (Ju et al. 2018). All these studies have suggested that water stress plays an important role in regulating the berry quality and controlling the substance synthesis in grape cultivation.

However, to date, the relationship between different degrees of water stress at different times and the balance of sugar and acid in 'Cabernet Sauvignon' has not been investigated in detail. This study mainly focused on the law of sugar accumulation and organic acid change in grapes under different development stages with different water stress levels, and understanding the mechanism of sugar metabolism under water stress, so as to provide a practical and theoretical basis for water-saving cultivation and quality improvement of wine grapes.

MATERIAL AND METHODS

Study area

The experiment was carried out at Yuquanying farm in the Ningxia agricultural reclamation group (38.28°N, 106.24°E) from May to October in 2017. The region's climate is typically continental and the soil is aeolian sandy, with an organic matter, alkali solution nitrogen, available phosphorus and available potassium content of 2 740, 22.35, 18.64, 68.44 mg/kg, respectively. Nine-year-old vines of *V. vinifera* 'Cabernet Sauvignon' were selected

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as the experimental material. The single Guyot training system was used, the row orientation is east-west with a spacing of 0.5 m × 3.0 m, the vineyard is equipped with a drip control system whose drip irrigation rate is 0.6 L/h.

Experimental design. Nine rows of vine were selected, 180 plants in each row, and water control valves were installed on the drip irrigation pipes at both ends of each row. In this experiment, three degrees of water treatment were set from setting to veraison separately, which were mild (CK, T1, T2), moderate (T3, T4, T5), and severe (T6, T7, T8), and within each group, a mild, moderate, and severe water stress treatment were set from post-veraison to maturation, and a total of nine groups were set for treatment, the data are shown in Electronic Supplementary Material (ESM) Table S1. All the treated vines bloomed (E-L 23) on May 25, 2017 (Coombe 1995), the time of veraison varied under the different water treatments. The forming light-light (CK), T1 and T2 colouring took place from July 30 [65 days after anthesis (DAA)] to August 4 (70 DAA), the T3, T4 and T5 colouring took place from July 23 (58 DAA) to July 29 (64 DAA), the T6, T7 and T8 colouring took place from July 25 (60 DAA) to August 1 (67 DAA), and all the treatments were collected on September 23 (E-L 38). Water stress began on June 8 (20 DAA) during the fruit setting. The irrigation amount was controlled by the drip irrigation time; the pre-dawn leaf water potential (Ψ_b) reflected the degree of water stress. All the treatments began to reach the set water potential range during the experiment at 40 DAA.

The pre-dawn leaf water potential (Ψ_b) values were measured every 10 days, the volume of the irrigation water (Supplementary Table S2) was adjusted to reach the set water range according to the rainfall and temperature during the experiment (Supplementary Figure S1), the pre-dawn grape leaves water potential (Ψ_b) is shown in Supplementary Figure S2.

Sampling began on June 25, 2017 (30 DAA), and was conducted every ten days until September 23, 2017 (120 DAA). Sampling was performed after measuring the pre-dawn leaf water potential. Pruning ten branches which have two berry bunches, and one to two buds left at the bottom were kept. The leaf and cluster were separated after weighing one hundred berries, part of which was frozen with liquid nitrogen and put in a refrigerator at –80 °C for determination of the AI, NI, SS and SPS activity. The remaining berries were used for the titratable acid content (TA) determination. Three technical replicates we used in the repeated measurements of the same sample.

Determination index and method

Pre-dawn leaf water potential. The healthy functional leaves in the middle of the new shoots of each treated vine were collected before dawn, put into plastic bags and brought back to the laboratory. The leaf water potential values were measured in a 3005-plant water potential pressure chamber (Soil Moisture Equipment Company, USA). Three plants were selected randomly for each treatment, and three leaves were taken from each plant.

Sugar composition in the berries. The total soluble sugar content was measured by using the anthrone-sulfuric acid method (Zhang, Qu 2003), and the high-performance liquid chromatography– evaporative light scattering detection (HPLC-ELSD) method was used to determine the sugar components in grape berries. The method of Li Mengge was used to extract the sugar components (Li 2015). One gram (1.0 g) grape berry powder and 25 mL ethanol (90%) were mixed and transfered into 25 mL volumetric flask. The mixture was ultrasonically extracted at room temperature for 30 minutes. The mixture was centrifuged at 10 000 rpm for 15 min, the supernatant was filtrated under a 0.22 μm filtration membrane, placed in a 2 mL centrifuge tube under 4 °C to save for futher determine. The chromatographic conditions: Prominence Ultra-Fast Liquid Chromatography (UFLC); the experiment was performed in a YMC-Pack Polyamine II chromatographic column (4.6 × 250 mm, 5 μm) with acetonitrile-water (75 : 25) as the mobile phase, the flow rate was 1.0 mL/min. The column temperature was kept at 30 °C, the injection volume was 10 μL.

The standard solution concentration was taken as the *X*-coordinate and the peak area as the *Y*-coordinate, the standard linear regression equation is shown in Supplementary Table S3, the chromatogram of the standard is shown in Supplementary Figure S3.

*Extraction and activity determination of the sucrose metabolism related enzymes.*The enzyme extraction and determination was performed with the method by (Gao 2006) with a moderate modification.

Enzyme extraction: 1.0 g of grape berry powder was weighed in a pre-cooled mortar, 2 mL of an extraction buffer was used for the ice bath grinding and then transferred to a centrifuge tube, 3 mL of an extraction buffer was added to wash the mortar and

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then the contents were transferred to the centrifuge tube, which was centrifuged at 4 °C (10 000 rpm) for 20 minutes. The supernatant was transferred to the dialysis bag, placed in the dialysis buffer, dialysed for 24 h at 4 °C, the dialysed enzyme solution was transferred to a 5 mL centrifuge tube and stored in a refrigerator at 4 °C. The AI, NI, SS and SPS activities were determined.

AI activity determination: 0.97 mL of the reaction solution and 30 μL of the dialysed enzyme solution were added to the 1 mL reaction system. After 30 minutes in a water bath at 37 °C, the reaction was stopped in a boiling water bath for three min. 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added, boiled in water for fine min, then cooled to room temperature, and the optical density (OD) value was measured at 540 nm. A dead enzyme was added as a control.

NI activity determination: Similar to the AI activity determination method, the 1 mL reaction system was changed into a 0.95 mL reaction solution and 50 μL dialysed enzyme solution.

SS activity determination: 0.4 mL of the enzyme reaction solution, 0.1 mL of uridine diphosphate glucose (UDPG) and 100 μL of the oxidised enzyme solution were added to the reaction system to replenish the water to 1 mL. After 30 min at 37 °C, the reaction was stopped by a boiling water bath for 3 min. 2 mol/L NaOH of 0.1 mL was added, then boiled in a water bath for 10 min, cooled to room temperature, and 3.5 mL of 30% HCL and 1 mL of 0.1% phenol were added, which was kept in the water bath at 80 °C for 10 minutes, then cooled to room temperature, and the OD value was measured at 480 nm. Distilled water was used instead of UDPG as the control.

SPS activity determination: Similar to the SS activity determination method, 10 mmol/L of fructose-6-phosphate (F-6-P) was used to replace the fructose in the enzyme reaction solution.

Organic acids. The titratable acidity was determined using standardised 0.1 N NaOH (end-point pH 8.2); the method of Gao (GAO 2004) with a moderate modification was used for the extraction and determination of the organic acids.

Data analysis. The statistical analysis was performed using Microsoft Excel 2010, a two-way analysis of variance and, subsequently, Tukey's test were performed to analyse the data, the differences were considered statistically significant at $P < 0.05$ using Data Processing System v15.10 (Hangzhou Ruifeng Information Technology Co. LTD., Hangzhou, China). Origin 9.0 and Sigmaplot 12.5 software (Systat Software, San Jose, CA, USA) were used to plot the results.

RESULTS AND ANALYSIS

Effects of water stress on the sugar composition in the grape berries

Effects of water stress on the fructose content in the grape berries. The fructose content in the berries from setting to harvesting showed a general trend of increasing rapidly at first, then slowly and steadily, as shown in Supplementary Table S4 in ESM. At 60–80 DAA, the fructose content in the berries with the severe treatment was significantly higher than the CK treatment, while at 80 DAA, the fructose content in the berries with the moderate treatment was also slightly higher than the CK treatment, indicating that short-term water stress from setting to veraison is conducive to the accumulation of fructose. At 90–120 DAA, the fructose content gradually increased and levelled off, among which the T1, T2 and T3 treatments were generally lower than the CK treatment, while the T4, T5, T6 and T7 treatments were generally higher than the CK treatment, indicating that an appropriate amount of water stress is conducive to the accumulation of fructose. The T8 treatment, which was treated heavily from setting to maturity stage, was always lower than the CK treatment at the post-veraison to maturity stage, indicating that long-term severe water stress is not conducive to the accumulation of fructose.

Effects of water stress on the glucose content in the grape berries. The trend in the glucose content change in the berries from setting to harvesting is basically consistent with the fructose, the overall trend is upward (Table S5 in ESM). At 60–70 DAA, the glucose content with the heavy treatment was significantly higher than the CK treatment, indicating that the heavy treatment from setting to veraison is conducive to the accumulation of glucose. At 90–120 DAA, the glucose content gradually increased and levelled off. The glucose content of the T1, T2 and T8 treatments were generally lower than the CK treatment, while the glucose content of the T4, T5, T6 and T7 treatments were generally higher than the CK treatment, indicating that a too light or excessive water treatment does not improve the glucose content.

Effects of water stress on the sucrose content in the grape berries. The variation trend in the sucrose content in grapes from setting to vintage also presents

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an overall increasing trend with the different treatment degrees (Table S6 in ESM). Except that the moderate treatment of 70 DAA was less than the CK treatment, the moderate and severe treatment was higher than the CK treatment at the other times, indicating that the moderate and severe treatment from setting to veraison are conducive to the accumulation of sucrose in the berries, and the severe treatment was more significant higher. At 90–120 DAA, the sucrose content in the berries tended to gently change. Among them, the T1, T2, T3 and T8 treatments were generally lower than the CK treatment, while the T4, T5, T6 and T7 treatments were generally higher than the CK treatment, indicating that a too light or excessive water treatment does not improve the sucrose content in the berries.

Effects of water stress on the total soluble sugar content in the grape berries. The total soluble sugar content from setting to harvest showed an overall increasing trend under the different treatment degrees (Table S7 in ESM). At 60–70 DAA, the moderate treatment was significantly higher than the CK treatment, indicating that the moderate treatment from setting to veraison is beneficial for the total soluble sugar accumulation. AT 90–120 DAA, the total soluble sugar content gradually increased and levelled off, the T4, T5, T6 and T7 treatments increased by 8.2%, 7.5%, 5.6% and 3.8% compared with the CK treatment at 120 DAA, respectively. At 60–120 DAA, the T8 treatment is always lower than the CK treament, indicating that an appropriate water treatment is conducive to increasing the total soluble sugar content, while the long-term excessive heavy treatment is contrary to this.

To sum up, the content of three sugars in the T4 treatment was significantly higher than the CK and other treatments at 120 DAA, and the T5, T6 and T7 treatments were also significantly higher than those in the CK treatment, which was consistent with the results of the total soluble sugar, indicating that the T4, T5, T6 and T7 treatments increase the sugar content in the berries. The sugar content of the T1 and T2 treatments at 90–120 DAA, which were treated lightly from setting to veraison and treated moderately and severely from post-veraison to maturity were substantially lower than the CK treatment, indicating that water stress in the early stage has a greater effect than that in the later stage. The T8 treatment, which was heavily treated from setting to maturity, was significantly higher than the CK treatment at 20–70 DAA and lower than the CK treatment at 110–120 DAA, indicating that the long-term water stress is not conducive to the accumulation of sugar in the berries. Therefore, a proper water treatment in the early stage is beneficial to control the sugar accumulation in the berries.

Effects of water stress on the activity of the sucrose-metabolising enzymes. During the growth and development process of the grape berries, the acid invertase activity of the grape berry firstly showed an increasing trend and then a decreasing one in a fluctuating manner (Figure 1A). Among the treatments, the acid invertase activity increased rapidly at 40–70 DAA and reached a peak at 70 DAA. The acid invertase activity was highest under the severe treatment, followed by the moderate treatment, and the CK treatment showed the lowest activity, indicating that water stress at the early stage of fruit ripening improves the acid invertase activity. At 90–110 DAA, the acid invertase activity in each treatment decreased and tended to be flat. In the maturity stage at 110 DAA, the T3, T4, T5, T6 treatments were higher than the CK treatment, which increased by 8.7%, 11.5%, 13.0% and 2.6%, respectively, indicating that the T3, T4, T5, T6 treatments improve the acid invertase activity. The T8 treatment was higher than the CK and other treatments at 40–70 DAA, and significantly lower than the CK treatments at 90–110 DAA, indicating that short-term water stress increases the acid invertase activity, while excessive water stress over a long period is contrary to this.

The neutral invertase activity in the growth and development process of the grape berries increased slowly at first and then tended to be stable (Figure 1B). Among them, at 40–60 DAA, the mild and moderate treatment showed a slow upward trend, while the severe treatment decreased, indicating that the neutral invertase is more sensitive to water stress in the early stage; at 70–110 DAA, it fluctuates and stabilises. At 90 DAA, except for the T3 and T8 treatments, the neutral invertase activity of all the other treatments were significantly higher than the CK treatments; at 100–110 DAA, the neutral invertase activity of the T4 and T5 treatments was higher than the CK treatment, and the difference between the T4 and CK treatments is extremely significant. These results indicate that the T4 and T5 treatments improve the neutral invertase activity. The neutral invertase activity in the T7 and T8 treatments were significantly

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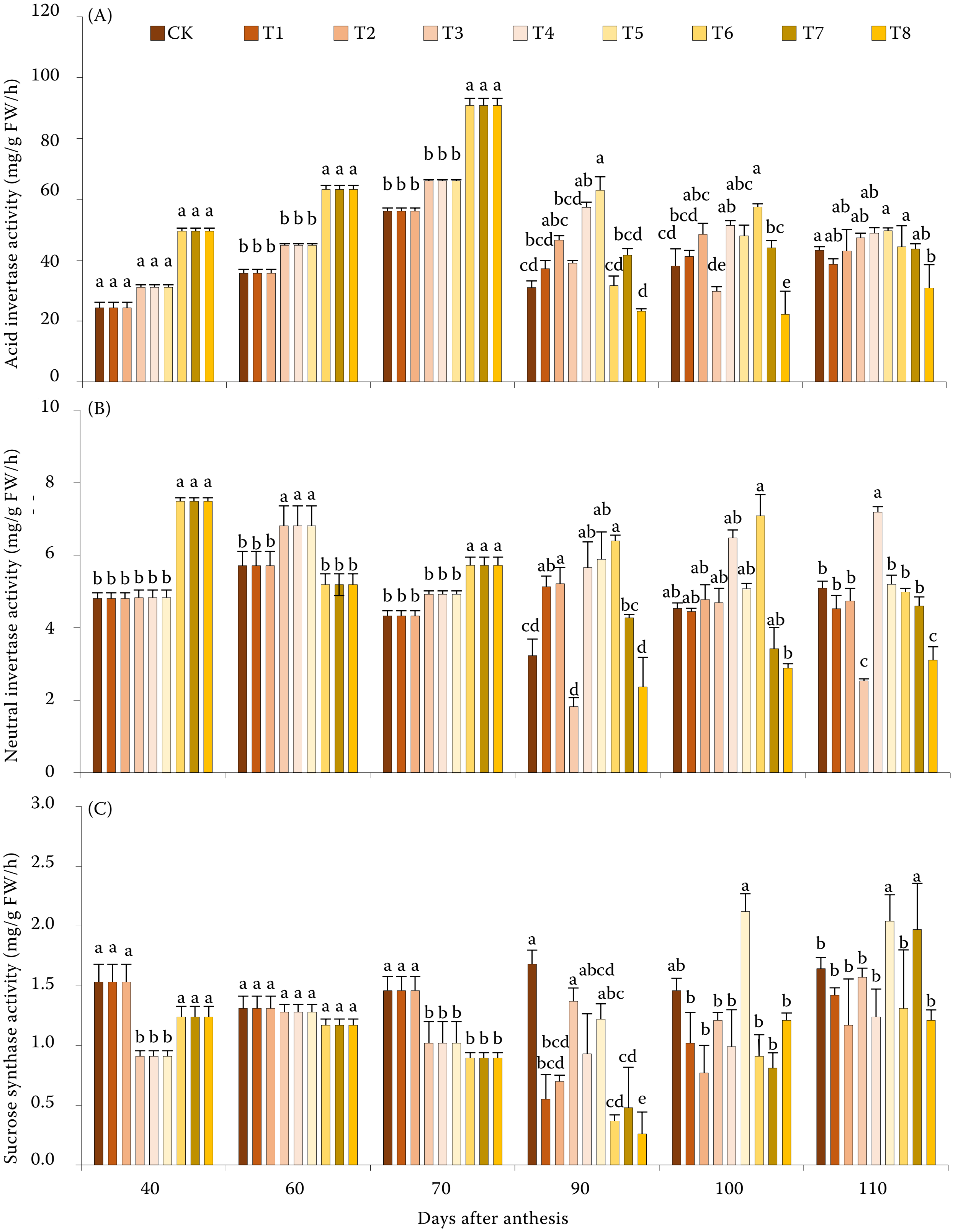

Figure 1. Effect of the water stress on the AI, NI, SS and SPS activity in the grape berries

The data are represented by mean ± SD from three independent experiments

Different letters indicate significant differences ($P < 0.05$) between the sample points within a single variety, as determined by a one-way analysis of variance followed by Tukey's test, using SPSS statistical software

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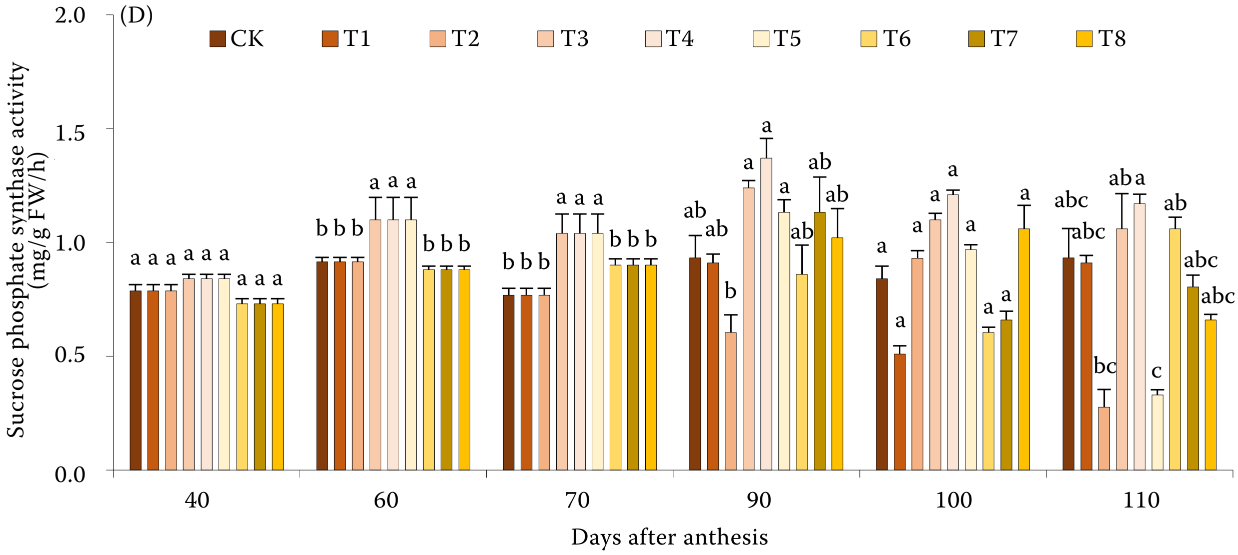
Figure 1 to be continued



lower than the CK treatment at 100–110 DAA, especially the T8 treatment, indicating that excessive water stress reduces the neutral invertase activity.

The sucrose synthase activity in the grape berry growth and development remained at a low level, which first declined and then fluctuated, but overall increased (Figure 1C). Among them, it shows a gradually decreasing trend at 40–70 DAA; at 90–110 DAA, there is an upward fluctuating trend. At 40–90 DAA, except for the T3 and T5 treatments at 90 DAA, all the other treatments showed a gradual decline, and the sucrose synthase activity of the CK treatment was higher than the other treatments. At 90 DAA, the sucrose synthase activity of the CK treatment reached a peak of 1.68 mg/g FW/h. At 100–110 DAA, except for the T5 treatment at 100 DAA, the sucrose synthase activity of the T5 and T7 treatment was significantly higher than the CK treatment, and all the other treatments were significantly lower than the CK treatment.

The sucrose phosphate synthase activity in the growth and development process of the grape berries always maintained a low level, and the overall level tends to be stable (Figure 1D). At 40–70 DAA, the treatment group was substantially higher than the CK treatment, but the difference was not significant. At 90 DAA, the sucrose phosphate synthase activity of the T3, T4, T5, T7 and T8 treatments was higher than the CK treatment, and the maximum value of the T4 treatment was 1.37 mg/g FW/h. At 100–110 DAA, the sucrose phosphate synthase activity of the T3 and T4 treatments was higher than the CK treatment. The sucrose phosphate synthase activity of the T8 treatment was higher than the CK treatment at all times except at 110 DAA, and had a peak value (1.06 mg/g FW/h) at 90 DAA.

Correlation analysis of the sugar components and the sucrose metabolisation-related enzyme activities in the grape berries. As shown above, water stress has an obvious influence on both the sugar components and metabolisation-related enzyme, a correlation analysis was conducted to further explore the relationship between the changes in the different sugar components and the variation in the metabolic enzyme activity.

During the grape berry ripening process, the AI is positively correlated with the sugar components of the grape fruits. Compared with the CK treatment, the T1, T2, T4 and T5 treatments increased the correlation between the sugar components and the AI in the berries, while the T3, T6, T7 and T8 treatments decreased the correlation between the sugar components and the AI in the berries (Table 1). It can be concluded that the AI activity is closely related to the accumulation of fructose and glucose in the grape berries.

There was a negative correlation between the NI and grape sugar components. Among them, the sugar components of the berries treated with T7 and T8 showed a significant negative correlation with the NI, while only the sugar components of the berries treated with T5 showed a positive correlation with the NI. It can be concluded that the activity of NI has no significant effect on the accumulation of the sugar components in the berries.

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Table 1. Correlation between the sugar components and enzyme activity related to the sucrose metabolism in the grape berries

Sucrose metabolism-related enzymes	Sugar component	Treatments								
		CK	T1	T2	T3	T4	T5	T6	T7	T8
AI	fructose	0.323	0.411	0.648	0.018	0.495	0.502	–0.139	–0.120	–0.340
	glucose	0.332	0.413	0.662	0.015	0.472	0.498	–0.142	–0.139	–0.333
	sucrose	0.392	0.418	0.798	–0.199	0.250	0.267	–0.283	–0.351	–0.467
NI	fructose	–0.348	–0.433	–0.226	–0.741	0.481	–0.207	–0.416	–0.828*	–0.867*
	glucose	–0.382	–0.454	–0.244	–0.748	0.519	–0.195	–0.429	–0.834*	–0.873*
	sucrose	–0.363	–0.458	–0.285	–0.620	0.594	–0.094	–0.313	–0.765	–0.915*
SS	fructose	0.515	–0.319	–0.700	0.706	0.013	0.733	–0.340	0.143	–0.224
	glucose	0.535	–0.294	–0.705	0.722	0.066	0.783	–0.360	0.140	–0.257
	sucrose	0.513	0.124	–0.726	0.785	0.040	0.819*	–0.146	0.339	–0.322
SPS	fructose	0.496	–0.057	–0.577	0.625	0.752	–0.341	0.284	0.153	0.321
	glucose	0.486	–0.049	–0.575	0.646	0.742	–0.361	0.274	0.155	0.343
	sucrose	0.434	0.199	–0.474	0.400	0.740	–0.479	0.192	0.083	0.627

*Significat correlation at $P < 0.05$, Tukey's HSD test

AI – acid invertase; NI – neutral invertase; SS – sucrose synthase; SPS – sucrose phosphate synthase; CK – forming light-light

SS was positively correlated with the sugar components of the grape berries. Compared with the CK treatment, the T3 and T5 treatments increased the correlation between the sugar components and the SS-s, while the T1, T2, T4, T6, T7 and T8 treatments decreased the correlation between the sugar components of the berries and the SS-s. It can be concluded that the activity of SS-s is mainly related to the sucrose accumulation.

The SPS was positively correlated with the sugar components of the grapes. Compared with the CK treatment, the T3 and T4 treatments increased the correlation between the sugar components and the SPS, the T8 treatments increased the correlation between the sucrose and the SPS, and the T1, T2, T5, T6, T7 and T8 treatments decreased the correlation between the sugar components of the grapes and the SPS. It can be concluded that the SPS is the key enzyme for the sucrose accumulation.

Effects of water stress on the organic acid content in the grape berries

Effects of water stress on the tartaric acid content during the grape berry development. The tartaric acid content in the grape berries under different water stress treatments showed an increasing trend firstly, then a rapidly decreasing and gradually stable one (Figure 2A). It showed a downward trend under moderate water stress at 20–40 DAA, while the tartaric acid content in the other treatments showed an upward trend; 40–90 DAA, the tartaric acid content decreased rapidly under each treatment, and the results showed that the severe water stress during setting to veraison could inhibit the tartaric acid synthesis.

The tartaric acid content per berry under the different water stress treatments showed an overall increasing trend. From 20 to 40 DAA, the tartaric acid content rapidly increased and then gradually increased (Figure 2B). The tartaric acid content per berry under the severe water stress was significantly lower than that under the mild and moderate water stress; 90–120 DAA, compared with the CK treatment, the tartaric acid content in the other treatments was lower; the highest tartaric acid content was in the CK treatment at 110 DAA (9.54 mg per berry), followed by the highest tartaric acid content in the T4 treatment, which was 8.95 mg per berry.

Effects of water stress on the change in the malic acid content in the grape berries during the development process. The malic acid content in the grapes under the different water stress treatments showed significant differences with an increasing trend firstly, then a decreasing rapidly and decreasing slowly one (Figure 3A). The malic acid content reached a maximum at 40 DAA under all the treatments, among which the malic acid content was the lowest (12.04 g/L) under the severe

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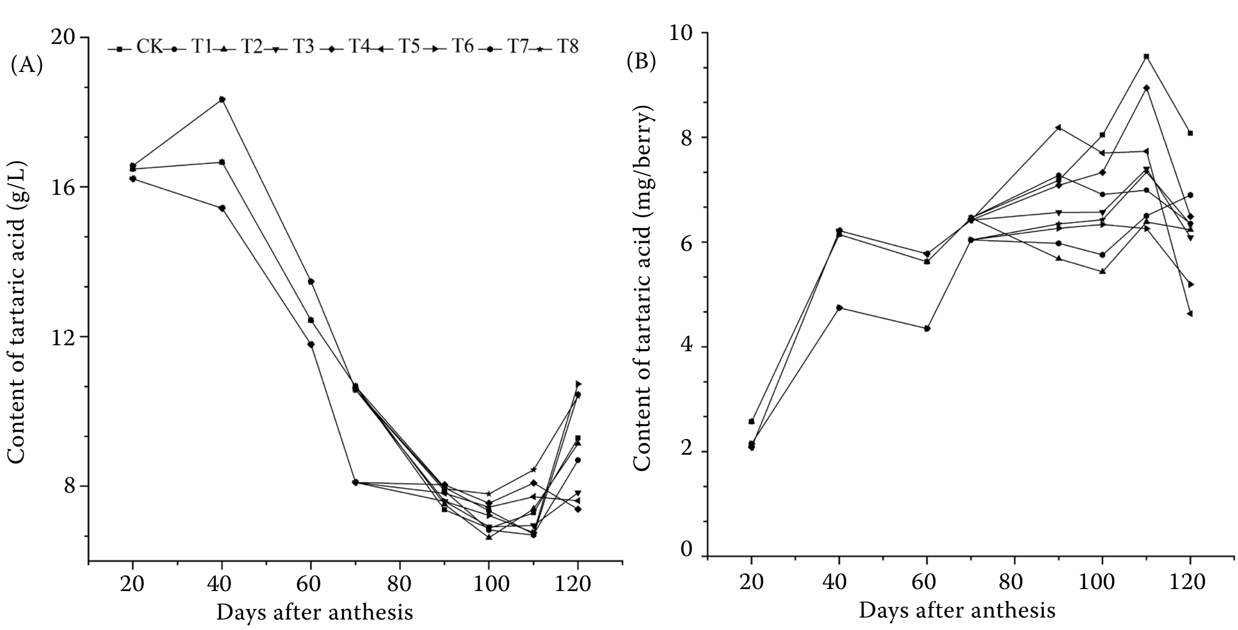

Figure 2. Effect of the different water stress treatments on the tartaric acid content in the Cabernet Sauvignon grape berries

(A) change in the tartaric acid content during the grape growth and development; (B) change in the tartaric acid content per berry during the grape growth and development

The data are represented by mean ± SD from three independent experiments

water stress; the malic acid content in the berries under the moderate water stress (T3, T4 and T5) decreased the most significantly, with an average decrease of 12.36 g/L; the malic acid content increased slightly under the T5 and T8 treatments during 90–110 DAA, which may be due to the

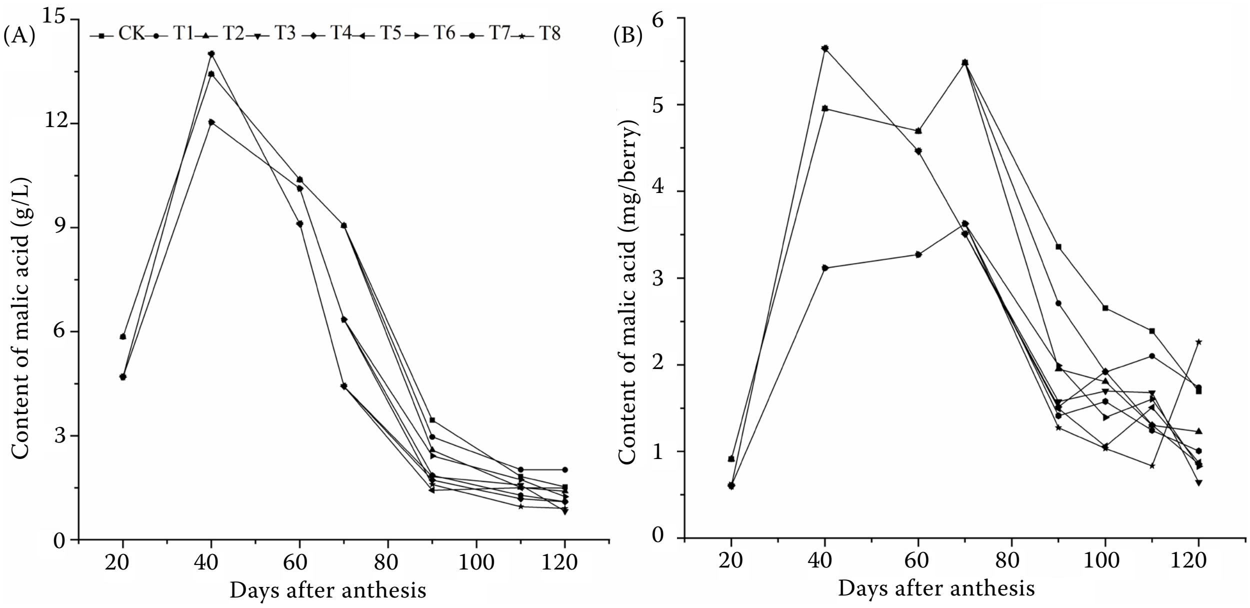

Figure 3. Effect of the different water stress levels on the malic acid content of the Cabernet Sauvignon grape berries

(A) change in the malic acid content during the grape growth and development; (B) change in the malic acid content per berry during the grape growth and development

The data are represented by mean ± SD from three independent experiments

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severe fruit shrinkage caused by the severe water stress at post-veraison.

The malic acid content per berry under different water stress showed an increasing trend at first, then a decreasing rapidly and decreasing slowly one (Figure 3B). At 20–40 DAA, the malic acid content per berry showed a large increase under the mild and moderate water stress and a small increase under the severe water stress, indicating that severe water stress inhibits the accumulation of malic acid per berry before veraison; the content of the malic acid per berry decreased significantly under the moderate water stress, while the malic acid content per berry did not change significantly under the other treatments. At 70–90 DAA, the malic acid content per berry of each treatment decreased rapidly, the CK treatment had the highest malic acid content which was 2.39 mg per berry at 110 DAA, and the other remaining water stress treatments all decreased compared with the CK treatment.

Effects of water stress on the change in the citric acid content in the grape berries during the development process. The citric acid content in the grape berries under the different water stress showed an overall increasing trend at first, followed by a decreasing and stabilising one, moreover, the citric acid content increased in all the treatments during 20–60 DAA, but the citric acid content has the biggest increase under the severe water stress, which was 0.42 g/L; the increase in the citric acid under the mild and moderate water stress was the same, which was 0.33 g/L; at 60–100 DAA, the citric acid content in the berries continuously decreased; at 90–110 DAA, the citric acid content in the berries increased under all the treatments except for the CK, T3 and T4 treatments; at 110 DAA, the citric acid content was the highest under the T1 and CK treatments, but there was no significant difference in the other treatments (Figure 4A).

During the whole growth period, the change in the trend of the citric acid content per berry was basically the same as that of the tartaric acid content.

Effects of water stress on the titratable acid (TA) content in the grape berries. The TA content showed a different declining degree in the process from setting to harvest (Figure 5). Among them, the TA content decreased rapidly at 40–70 DAA, and gradually levelled off at 100–120 DAA. During 40–120 DAA, the TA content of the T8 treatment was generally higher than the CK treatment, indicating that severe stress hinders the degradation of the TA in the grape berries; at 120 DAA, the TA content of the T3, T4, T5, T6 and T7 treatments compared with the CK treatment decreased by 2.6%, 1.2%, 2.2%, 1.8% and 13.4%, respectively, indicating that a proper water treatment promotes the degradation of the total acid content.

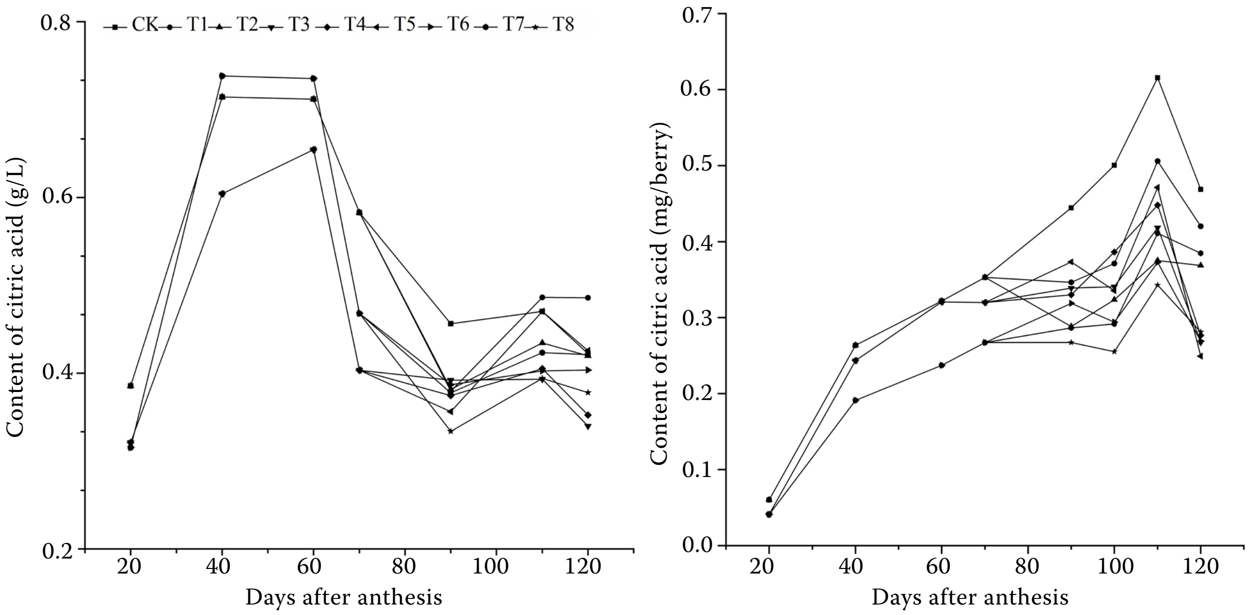

Figure 4. Effect of the different water stress levels on the citric acid content of the Cabernet Sauvignon grape berries (A) the change in the citric acid content during the grape growth and development; (B) the change in the citric acid content per berry during the grape growth and development

The data are represented by mean ± SD from three independent experiments

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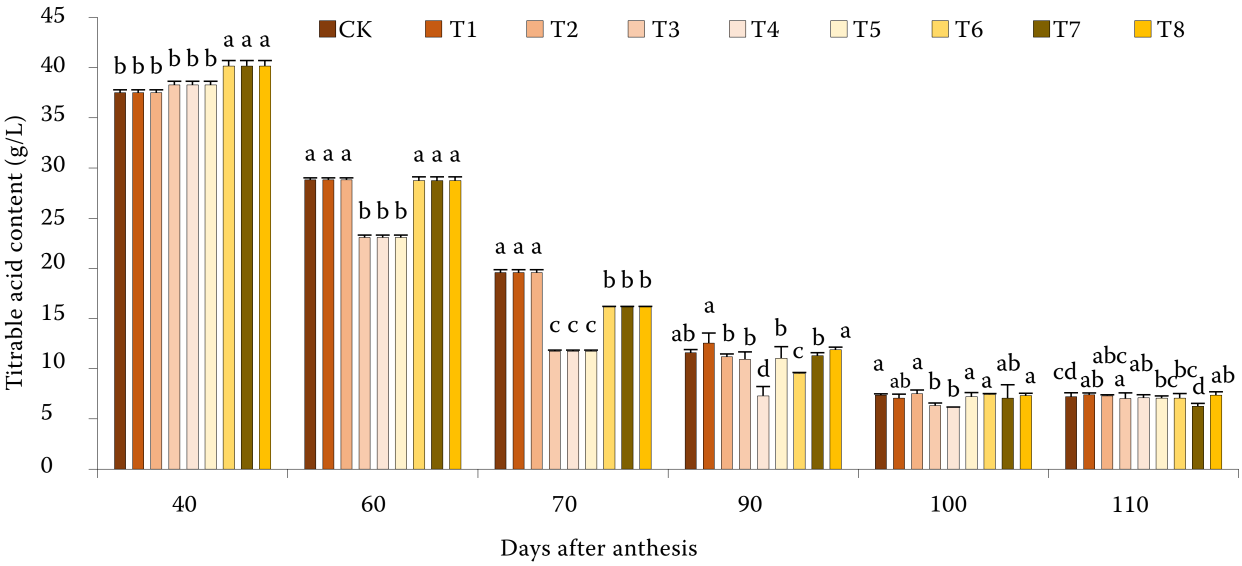

Figure 5. Effect of the water stress on the organic acid content of the grape berries

The data are represented by mean ± SD from three independent experiments

Different letters indicate significant differences ($P < 0.05$) between the sample points within a single variety, as determined by a one-way analysis of variance followed by Tukey's test, using SPSS statistical software

DISCUSSION

When plants are subjected to drought stress during their growth and development stages, their physiological metabolism will change (Per et al. 2017). Water stress can promote an increase in the total soluble sugar content in grapes, but severe water stress can reduce the total soluble sugar content in grapes, the results showed that an appropriate level of water stress was beneficial to the accumulation of the total soluble sugar content in grape berries, while the long-term severe water stress caused irreversible damage to the plants, thus reducing the quality of the fruit. The main sugars accumulated in grapes are fructose, glucose and a small amount of sucrose (Davies, Robinson 1996). In this study, the content of the three sugars increased in an "S" curve and reached the maximum value during the fruit ripening period, indicating that the water stress did not change the accumulation pattern of the sugar components in the grape berries. A large number of previous studies have shown that an appropriate water stress level can increase the sugar component of fruit (Peterlunger et al. 2002; Castellarin et al. 2007). This study found that, during the harvest period, the content of the three sugars in the T4 treatment was significantly higher than that in the CK and other treatments, and that in the T5, T6 and T7 treatments was also higher than that in the CK treatment, which was consistent with the results of the total soluble sugar, indicating that the T4, T5, T6 and T7 treatments increase the content of the sugar components in the grape berries.

Studies have found that, on the basis of deficit irrigation, the fruit growth rate of pear trees can increase after rehydration (Cui et al. 2009). In this study, the T6, T7 and T8 treatments were treated severely from setting to veraison and were treated mildly, moderately and severely from post-veraison to harvesting stage, respectively. The content of the sugar components of the T6 and T7 treatments was still higher than the CK treatment after rehydration, while the T8 treatment was lower than the CK treatment, indicating that the T8 treatment caused unrecoverable damage to the grape berries. The time and intensity of the water deficits influence the degree of metabolism occurring in the berry (Chaves et al. 2010). The T3, T4 and T5 treatments, which were all moderately treated from setting to veraison, were respectively moderately and severely treated from post-veraison stage to harvesting stage, which could increase the sugar content of grape berries, indicating that the T4 and T5 treatments were beneficial to the accumulation of the grape berry sugar. In the T1 and T2 treatments, which were treated lightly from setting to veraison and were treated moderately and severely from post-veraison to harvest period, the content of sugar component was generally lower than

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the CK treatment at 90~120 DAA, the reason may be the I growth stage (growth phase) is the critical period that determines the split, the water stress effect on the II and III growth period (lag phase; ripening and growth phase) was not significant compared with the I growth period (Pérez-Pérez et al. 2014). The results showed that both light and heavy water stress treatments were not conducive to the accumulation of the sugar components in the grape berries.

Sucrose metabolisation-related enzymes play an important role in the degradation of sucrose and are closely related to the unloading of the phloem and the accumulation of cellular sugar. Sucrose synthases (SPS + SS-s) promote sucrose accumulation, and sucrose decomposers (SS-s + AI + NI) promote hexose accumulation (Klann et al. 1996; Hongyan et al. 2005). This study found that the activity of AI under the T3, T4, T5, T6 and T7 treatments was higher than the CK treatment at 110 DAA, indicating that water stress could significantly improve the AI activity. The AI activity was significantly higher than the NI activity, and the water stress increased the activity of two invertases, indicating that the AI activity was closely related to the strength of the water stress. Under water stress, the activity of SS-s in the berries from setting to ripening was always lower than the CK treatment, but the activity of the T5 and T7 treatments at 120 DAA was higher than the CK treatments. Between 20 and 30 days before physiological maturation, the activity of invertase (AI) increased significantly, while inversely, the SPS and SS had lower activities in the papaya mesocarp (Zhou et al. 2018). In this study, it was found that water stress could increase the activity of the SPS over the entire berries' growth period, and the activity increase of the SPS in the late stage of veraison was greater than that in the early stage of veraison.

During the development of the grape berries, the organic acid content in the berries first increased and then decreased significantly, in which malic acid was the main component that decreased. Inside the cell, malic acid is formed from phosphoenolpyruvate (PEP) through the activities of phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) (Sweetman et al. 2009), and cytosolic malate is transported and sequestered in the vacuole (Schulze et al. 2002). Malate can be degraded by the reversible reaction of MDH and nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP-ME) (González-Agüero et al. 2016). In grapes (*V. vinifera* L.), the malic acid content always decreases as the light intensity increases and as the water supply decreases (Zheng et al. 2018). Tartaric and malic acids, which are the main organic acids that generally account for 69 to 92% of all the organic acids in grape berries and leaves (Kliewer 1966), rapidly decrease during the fruit ripening (Liang et al. 2011). Studies have proven that water stress can increase the organic acid content in grape berries (López et al. 2009; Cholet et al. 2016). According to the current analysis, the tartaric acid content per grape was higher than that of the other two acids (citric acid and malic acid). The tartaric acid and citric acid content increased under water stress, while the malic acid content first increased and then decreased during the second growth period, the tartaric acid content in the grape berries remained unchanged, but the malic acid content decreased rapidly. Citric acid is mainly involved in respiration and gluconeogenesis as a substrate (Cercós et al. 2006). The amount of tartaric acid per berry is stable after veraison, the decrease in the concentration during ripening is due to the increase in the volume (DeBolt et al. 2008), it was once again proven that tartaric acid was synthesised and accumulated during the whole growth and development of the grape berries, but not degraded and utilised, mainly because it did not participate in the main biochemical metabolism process.

During the growth and development of grape berries, various organic acids in the berries are accumulated, but the organic acid content was different under the different water stress treatments. Based on the analysis, it can be seen that light treatment from setting to veraison is beneficial to the synthesis of various organic acids, while heavy water stress will inhibit the synthesis of organic acids. This is due to applying the water stress before the veraison, which reduces the activity of the malic acid anion channel and malic acid operating protein on the vacuole membrane, blocks the release of malic acid from vacuole into cytoplasm, and further affects the expression of malate and the malate dehydrogenase related genes. The grape berry organic acid content directly affects the taste and aroma of a wine, and too low of an acid content will make the wine body unbalanced, resulting in quality degradation (Ju et al. 2018). In order to preserve the organic acid content in the grapes, a light or moderate water stress treatment should be applied from setting to veraison. Combined with the effects of the water stress on the grape berry quality, the T5 and T6 treatments had moderate titratable acid contents

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in nine water stress treatments, which met the requirements of wine production.

CONCLUSION

The results of this study demonstrate that:

– Water stress can increase the total soluble sugar, fructose, glucose and sucrose content in grape berries. Pre-veraison is an important period to synthesise the organic acid, and the malic acid content gradually decreases after veraison.
– Light water stress before veraison is conducive to the organic acid synthesis, while a heavy treatment inhibits the organic acid synthesis. The T5 and T6 treatments had moderate titratable acid contents which meet the requirements of wine production, while the T8 treatment will hinder the TA degradation.
– All the treatments increased the AI, NI and SPS activities over the whole grape growth period, and the AI activity was higher than the NI and SPS activity on the whole. All the treatments reduced the SS-s activity before veraison and increased the SS activity at post-veraison.

To sum up, a moderate treatment is suitable from setting to veraison, and a moderate and severe treatment are suitable from post-veraison to harvesting, because these treatments were conducive to the sugar transport and accumulation, as well as improving the fruit quality.

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