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Effects of Water Stress on Resveratrol Accumulation and Synthesis in ‘Cabernet Sauvignon’ Grape Berries

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Abstract: Resveratrol (3, 4', 5 trihydroxy stilbene) is a natural phytoalexin produced by plants in response to biotic and abiotic stresses. It is well known for its cardio-protective, anticarcinogenic, and antioxidant properties. This study characterized physiological and molecular changes in resveratrol synthesis exposed to two levels of water stress at distinct grape berry developmental stages. Physiological data were measured to assess the berry quality. We used high-performance liquid chromatography to study enzyme activity and qRT-PCR to assess the expression levels of genes involved in resveratrol synthesis. The berry development was suppressed under water stress, while the content of total polyphenol, especially resveratrol was enhanced. Related enzymes and genes regulate the changes in resveratrol in plants. Water stress improved the enzyme activities of PAL (phenylalanine ammonia-lyase) and STS (stilbene synthase) after veraison. Moreover, the transcription factors *VvMYB14*, *VvMYB15* and resveratrol synthesis-related genes are also changed. Expression of *Vv4CL* and *VvMYB15* were upregulated during the pre-reversion stage, whereas *VvPAL* and *VvSTS* increased throughout development. As the co-substrate of *VvSTS*, *VvCHS* decreased during the berry development. Our study demonstrates that water stress regulates resveratrol synthesis through enzymatic activities and the gene expression of PAL and STS.

Keywords: water stress; resveratrol; grape; STS



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1. Introduction

Grapes constitute one of the primary sources of phenolic compounds among different fruits. They are a rich source of bioactive molecules, including stilbene, flavonols, proanthocyanins, and anthocyanins, which are highly effective against cardiovascular diseases [1]. Resveratrol (3, 5, 4'-trihydroxystilbene), one of the main bioactive components of the grape, is a natural phenylpropanoid that accumulates under the stimulation of environmental factors (UV light, wounding, pathogen) [2]. This phytochemical has attracted considerable attention for its health-promoting effects through anti-inflammatory, anti-cancer, immune-enhancing, anti-lipid peroxidation, and anti-apoptotic effects [3,4]. Resveratrol is a member of the flavonoid family, consisting of two aromatic rings joined by a methylene bridge [5,6]. Generally, there exists two geometric isoforms (trans and cis) of resveratrol. Trans-resveratrol is the most stable iso-form but can be transformed into cis-resveratrol by Solar and UV irradiation [7]. Moreover, there is a substantial proportion of resveratrol in its glycoside, called piceid, except trans- and cis-resveratrol [8]. Glycosyltransferases produce glycosidic forms. Resveratrol is synthesized via the phenylpropanoid/malonate pathway involving a series of enzymes such as phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL) and stilbene synthase (STS). Among them, both STS and CHS belong to the type III polyketide synthase superfamily (PKSs). Although they share the same substrates, STS catalyzes the direct formation of the stilbene skeleton through a single reaction from three units of malonyl-CoA and one CoA-ester of a cinnamic acid derivative. CHS is responsible for the first committed step in the biosynthesis of flavonoid-type compounds [9,10]. At present, STS genes have been cloned from several

plant species. According to the investigation of the genome organization in the grapevine, the grapevine STS gene family consists of 48 members, consisting of at least 33 potential functional genes [11]. Structural genes and regulatory genes regulate the synthesis of resveratrol. Holl et al. [12] demonstrated that *VvMYB14* and *VvMYB15* were involved in the transcriptional regulation of stilbene biosynthesis in grapevine. Grape resveratrol biosynthesis depends on multiple factors including fungal and environmental stresses [13]. Grapes are often cultivated in semi-arid regions and subjected to prolonged droughts that limit berry growth [14]. Resveratrol is generally synthesized in plants in response to external stress and water is one of the critical environmental factors [15]. Water scarcity is a principal abiotic stress that negatively affects crops' metabolism, growth, and yield, including resveratrol [16]. However, little is known about variations in resveratrol levels in grape berries to changing environmental conditions, such as water stress or elevated air temperatures. Therefore, the major focus has been on assessing the effect of water stress on resveratrol synthesis in grape berries. In this research, we aimed to study the impact of water stress on grape compositions, particularly resveratrol biosynthesis. To uncover their relationships, we conducted an experiment that applied three water regimes. The berry composition, contents of resveratrol and resveratrol synthesis-related enzyme activities, and related structural genes were detected at several developmental stages, revealing the mechanism of water stress on resveratrol synthesis.

2. Materials and Methods

2.1. Plant Material and Experimental Treatments

Fifteen-year-old grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) were cultivated in sandy soil, with spacing at 0.5 m × 3 m with the row-oriented in a north–south direction at the vineyard in Yuquanying farm, Ningxia, China (latitude 38°14'25" N, longitude 106°01'43" E). Irrigation was applied through a single drip line per row of trees with two pressure-compensated drippers per tree with a nominal emitter discharge of 0.6 L·h^{−1}. Three parallel irrigation treatments were established according to the reference of Elman et al., and all drippers were within 5% of the prescribed rate [17]. The trial started on 20 June, 25 days after anthesis (DAA) and the pre-dawn leaf water potential was −0.3 MPa. A total of 8–10 mature leaves per treatment were measured every five days on the same day. Irrigation was applied to control (CK) vines to maintain pre-dawn leaf water potential (ϕpd) between −0.2 and −0.4 MPa throughout the season. T1 vines were watered to maintain an average pre-dawn leaf water potential between −0.4 MPa and −0.6 MPa. T2 vines needed a status above −0.6 MPa, which relates to a severe grapevine water deficit. Pre-dawn leaf water potential (ϕpd) was measured between 5:30 and 6:00 am with pressure chamber (Soil Moisture Equipment Co., Goleta, CA, USA) according to the method of Castellarin et al. [18]. The trial was set up with 11 rows in a randomized block design (60 vines/row). Each block consists of three lines, separated by one buffer line. Samples were taken from a row of 90 vines located in the center of the block. Berries were sampled after measuring the ϕpd (every 10 d from 30 to 120 DAA), and quickly frozen by liquid nitrogen and stored at −80 °C.

2.2. Measurements of Physiological Data and Stilbenes

Samples for berry quality measurements were weighed and pressed. Soluble solids were determined on a small aliquot of the juice with a hand-held refractometer. Soluble sugar was gauged by the anthrone-sulfate method. A total of 2 mL of the juice was used for testing titratable acidity. A sample of 30 berries was powdered for anthocyanin and total polyphenol analyses, the contents were measured by Castellarin et al. [18]. Veraison period was estimated by the color distribution (%) of berries, mainly counting pink and red fruits from the treated vines. The analysis of the stilbenes was performed according to the method of Souid et al. [7]. Each sample (berry skin) was extracted for 24 h in methanol and ethyl acetate (1/1, v/v; 1000 mg per 10 mL of organic solvent) at 25 °C in the dark. The suspension was centrifuged at 8000 rpm for 15 min. Each sample was filtered through a

0.45 µm PTFE membrane filter. Resveratrol was analyzed on a Prominence LC-20A HPLC system. Separation was achieved on AcclaimTM 120 C18 columns (250 mm × 4.6 mm, 5 µm) maintained at 30 °C using a mobile phase consisting of H₂O (A) and acetonitrile (B). The solvent gradient was performed in the following manner, 0 min (10% B), 5 min (17% B), 12 min (18% B), 22 min (20% B), 30 min (33% B), 45 min (38% B) and 58 min (100% B). For fluorometric detection, the maximum absorption wavelength of two trans-isomers (trans-resveratrol, and trans-piceid) was 306 nm and two cis-isomers (cis-resveratrol and cis-piceid) was 288 nm. The retention time of stilbenes is shown in Figure A1. The harvest date was fixed based on the ripening dynamics of the berries related to sugar concentration (mg/g), titratable acidity (g·L⁻¹) and pH (data not shown).

2.3. Measurements of Enzyme Activity

The activity of phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H), and 4-coumarate: CoA ligase, (4CL) were detected according to the methods of Knobloch et al. [19–21]. The activity of stilbene synthase referred to the method of Krzyzaniak, Negrel [22] with slight modification. Thirty berry samples were ground into powder and 1 g was extracted with 0.2 mM Tris-HCl (pH 7.5) and 10 mM ascorbic acid buffer (containing 1 mM EDTA, 10 mM β-mercaptoethanol pH 7.5), then centrifuged at 10,000 rpm for 10 min to obtain the supernatant. The reaction mixture contained 30 µL of 0.1 mM Tris-HCl (pH 7.5), 10 µL of 2.5 mM malonyl-CoA, Coumaroyl CoA and 50 µL of the extract. After centrifugation, the reaction was stopped by adding concentrated acetic acid and 10 µL taken for HPLC analysis.

2.4. RNA Extraction and Quantification of mRNA

The total RNA was extracted according to the RNeasy Pure Polysaccharide Polyphenol Plant Total RNA Extraction Kit DP441 (Apexbio, Co. Ltd., Beijing, China). Reverse transcription and purification were performed by the PrimerScriptTM RT reagent Kit with gDNA Eraser (Perfect Real time) kit. EF1 and Actin were selected as internal reference genes. Gene primers of PAL, 4CL, CHS, STS, Myb14 and Myb15 were designed with Primer5.0 and synthesized by Lanzhou Biosynthetic (Table A1). The RT-qPCR system was 25 µL: cDNA (100 ng·µL⁻¹) 1 µL, upstream primer and downstream primer were 0.5 µL, respectively, 2× UltraSYBR Mixture 12.5 µL, ddH₂O 10.5 µL. RT-qPCR was performed under the following conditions: 95 °C for 10 min; denaturation at 94 °C for 10 s, annealing at 54–56 °C for 30 s, 40 cycles; extension at 72 °C for 32 s. The templates were carried out with three replicates and averaged. The results were calculated by the method of $2^{-\Delta\Delta C_t}$.

2.5. Statistics

Data are expressed as average ± standard errors of three biological replicates. The statistical analysis was performed by Duncan's multiple range test of variance (ANOVA), using SPSS software version 17.0. Means were separated by Duncan's multiple range tests at $p < 0.05$.

3. Results

3.1. Weather Conditions and Leaf Water Potential

First, we measured the pre-dawn leaf water potential (ϕ_{pd}) to monitor the water status of the plant (Figure 1c). Differences in the pre-dawn leaf water potential (ϕ_{pd}) between three treatment vines were detected 40 days after anthesis (DAA). The ϕ_{pd} of CK were between −0.2 and −0.42 Mpa, and the value of the two treatments were in the range of −0.4~−0.61 and −0.6~−0.73, respectively. On days 65 and 85 DAA, the three groups of ϕ_{pd} increased due to the rainfall. The weather conditions recorded in the experimental area are reported in Figure 1a. During the water stress trial, the maximum and minimum temperature were 39 °C and 12 °C, respectively, and the average was 23.9 °C. The daily maximum temperature exceeded 38 °C for four days, with the highest temperature recorded (39 °C) 49 days after anthesis. There were few rainfall events before veraison (60DAA),

totaling 13.4 mm. However, the rainfall was abundant in the period from veraison to maturity (60–95 DAA), with almost 71 mm. However, each instance of sampling treatment was in a particular processing range.

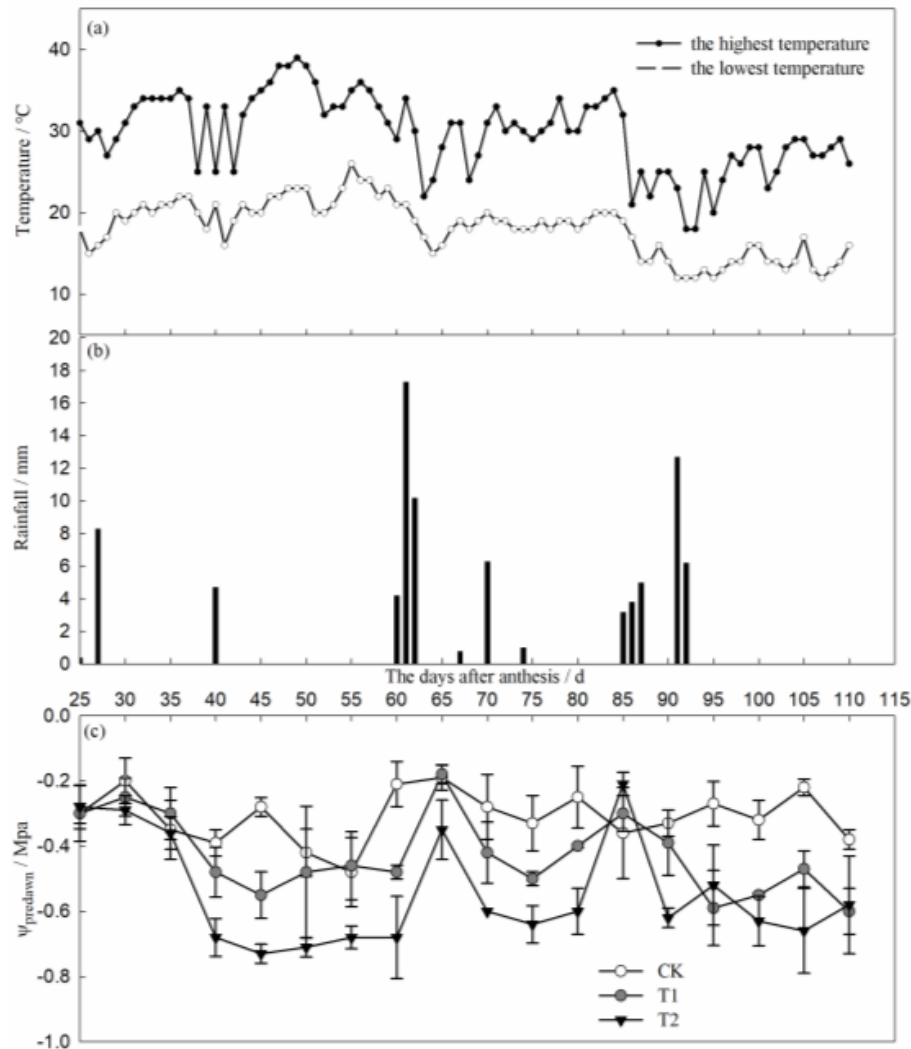


Figure 1. Weather conditions of the experimental field and the pre-dawn of leaf water potential of cabernet sauvignon. (a) air temperature (°C), (b) daily rainfall (mm), (c) pre-dawn of leaf water potential. CK Control ($-0.20 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.40 \text{ Mpa}$); T1, Treatment 1 ($-0.40 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.60 \text{ Mpa}$); T2 Treatment 2 ($\phi_{\text{predawn}} \leq -0.6 \text{ Mpa}$). Vertical bars indicate $\pm \text{SE}$.

3.2. Physiological Responses to Water Stress

The phenological period refers to the timings of cyclical or seasonal biological events. We recorded the phenological changes of the experimental grapes throughout the growth cycle. The budburst period was on 24th April, the full bloom period was on 25th May, the fruit setting period was on 25th May and the green fruit period was on 13th June. However, the veraison period of each treatment is different (the veraison period corresponded to the onset of the ripening period identified as the date of berry coloring begins to 50% veraison). The period of CK veraison was from 30 July (65 DAA) to 4 August (70 DAA), T1 was from 23 July (58 DAA) to 29 July (64 DAA) and T2 was from 25 July (60 DAA) to 1 August (67 DAA), indicating that water stress can change the phenological period of grapes. Berry compositions (berry weight, berry volume, sugar, titratable acidity, total polyphenols, and anthocyanin) were determined throughout development in both well-watered (CK) and water stress (T1, T2) treatments (Table 1). The data showed that water stress significantly

affected berry characteristics, sugar, and titratable acidity ($\text{g}\cdot\text{L}^{-1}$). During the development, the berry weight of treated plants was smaller, with a difference of up to 0.3 g at the harvest stage (Table 1). Berry volume was significantly affected in severe water deficit (T2) vines, with a 100 mm^3 reduction compared to CK. There was no significant effect of water stress on titratable acidity during veraison. Like berry weight, the contents of total polyphenols and anthocyanin were significantly affected by water stress. Water deficits increased their contents. Both the treated vines were higher at the harvest stage than in CK. Furthermore, the increase in the T1 group was more pronounced than in T2. Thus, water stress significantly affects berry compositions, and moderate water stress can improve berry quality.

Table 1. Berry characteristics and compositions of ‘Cabernet Sauvignon’ treated with different levels of water stress. **, ***, ns indicates significant differences at $p < 0.01$, 0.001 , or not significant. Different letters indicate significant differences, and “a–c” in the same column means $p < 0.05$. CK, Control ($-0.20\text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.40\text{ Mpa}$); T1, Treatment 1 ($-0.40\text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.60\text{ Mpa}$); T2 Treatment 2 ($\phi_{\text{predawn}} \leq -0.6\text{ Mpa}$).

Stages	Treatments	Berry Characteristics				Berry Compositions		
		Berry Weight	Berry Volume	Total Soluble Solids	Sugar	Titratable Acid	Total Polyphenols	Total Anthocyanin
		g	mm^3	%	mg/g	$\text{g}\cdot\text{L}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$
Green fruit (30 DAA)	CK	0.46 ^a	154.56 ^a	4.33 ^b	11.60	40.15 ^a	18.23	0.00
	T1	0.39 ^b	131.98 ^a	5.20 ^{a,b}	11.40	38.25 ^b	18.25	0.00
	T2	0.26 ^c	90.88 ^b	6.20 ^a	11.75	37.48 ^b	18.86	0.00
	ANOVA	***	***	**	ns	***	ns	ns
Veraison (70 DAA)	CK	1.00 ^a	215.95 ^a	15.00	91.56 ^b	19.56 ^a	11.44	0.11 ^b
	T1	0.86 ^b	235.06 ^a	14.60	116.93 ^a	18.32 ^b	11.4	0.34 ^a
	T2	0.64 ^c	167.33 ^b	14.07	93.60 ^b	16.16 ^c	12.58	0.15 ^b
	ANOVA	***	**	ns	**	***	ns	***
Maturity (110 DAA)	CK	1.16 ^a	275.54 ^a	20.64 ^a	204.35 ^a	7.36 ^a	6.29 ^b	0.77 ^b
	T1	1.09 ^b	273.45 ^a	20.09 ^a	199.96 ^a	6.19 ^b	8.49 ^a	0.82 ^b
	T2	0.83 ^c	173.64 ^b	19.16 ^b	191.23 ^b	7.32 ^a	7.66 ^a	0.95 ^a
	ANOVA	***	***	**	**	***	**	***

3.3. Effects of Water Stress on the Accumulation of Stilbenes

In this study, we compared the concentrations of *trans*- and *cis*-resveratrol and its two glycosides, *trans*- and *cis*-piceid, in Cabernet Sauvignon grapes from veraison to ripening (Figure 2). The results showed that the concentrations of *trans* and *cis* resveratrol and its two glycosides accumulated with time. Water stress significantly affected the accumulation of stilbenes. Compared with CK, T1 and T2 significantly increased *trans*- and *cis*-resveratrol contents. *Trans*- and *cis*-resveratrol contents in both treatments were low in the early stage but increased rapidly after 80 DAA, reaching a peak at 90 DAA. The content of *trans*- and *cis*-piceid increased slowly at prophase (60–80 DAA) and then rapidly from 90 DAA until the fruit ripened. In contrast to *trans*-, *cis*-resveratrol, both *trans*- and *cis*-piceid in CK were significantly greater than the treatment groups.

3.4. Effects of Water Stress on Activities of Resveratrol Synthesis-Related Enzymes in Grape Berries

We further investigated the changes in four main enzymes synthesizing the berries’ resveratrol. The enzymatic activity of four enzymes in berries exhibited a large increase in each treatment (Figure 3). The PAL activity of grape berries changed considerably during the ripening, showing a tendency to decline first and then increase. The activity of the treated vines was significantly higher than that of the CK after 80DAA. The activity of C4H in the T1 group was higher than in CK during 60–90 DAA. However, there was no significant difference in enzymatic activity between T1 and CK groups at maturity.

Like C4H, the enzyme activity of 4CL displayed a trend to grow first and then decline. Cumulative enzyme activity of 4CL was higher in both water stress treatments than CK and was most remarkable in T2 berries. Water stress treatments also increased the enzyme activity of STS. The activity of STS in the T1 and T2 groups was higher than in control during 60–70 DAA. Notably, the activity of STS in the T1 group was consistently higher than in the other groups throughout development and reached the maximum at 80 DAA. The results demonstrated that different degrees of water stress had different effects on resveratrol synthesis-related enzymes in different stages of the grape berries. The T1 group significantly increased the C4H and STS activities, while the T2 group enhanced the PAL and 4CL.

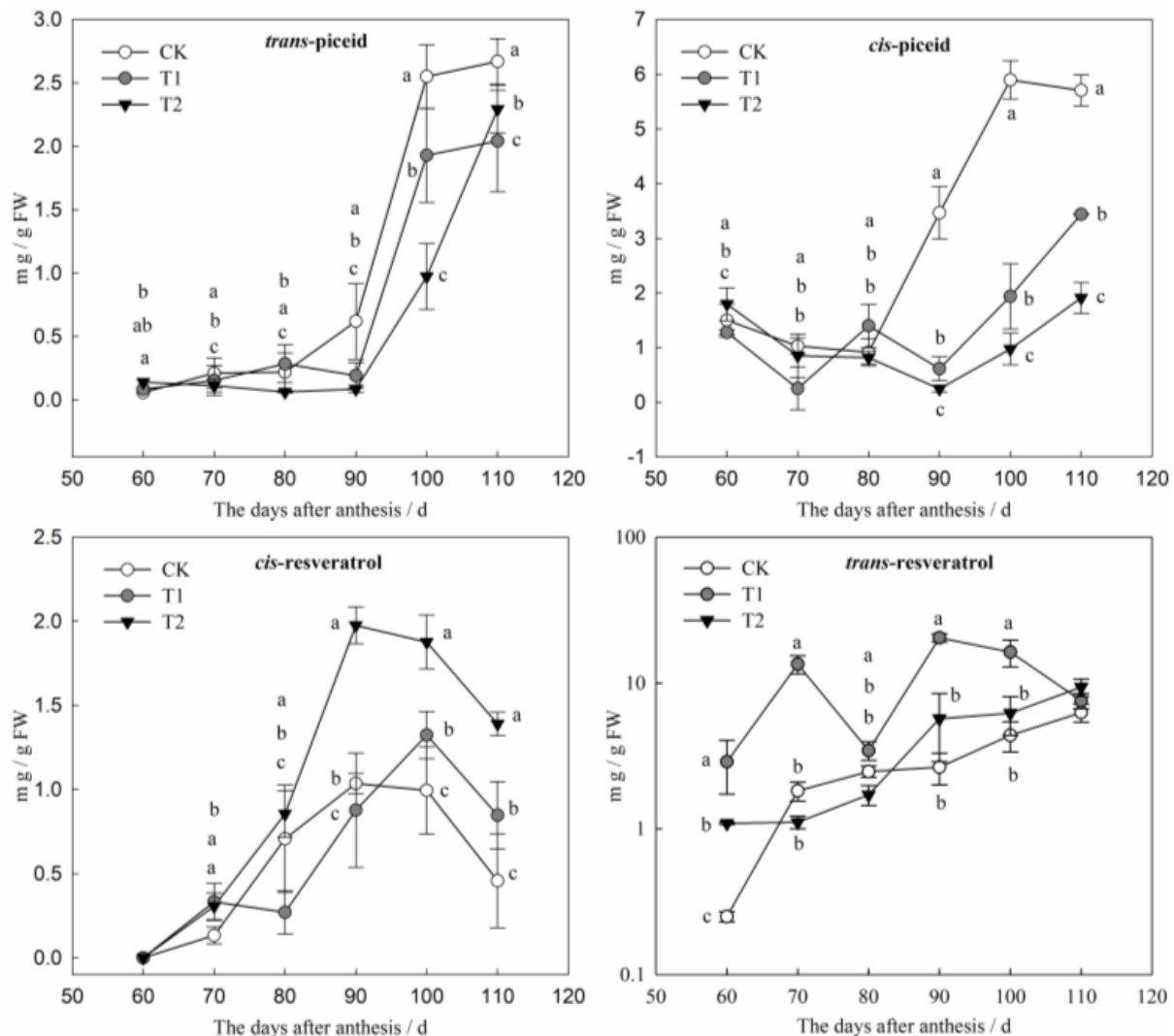


Figure 2. Variation in the concentration of resveratrol and its derivatives during berry development. The data are represented by mean $\pm$ SD. According to Duncan’s multiple range test (DMRT), values with different letters are significantly different at the 5% level. CK, Control ($-0.20 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.40 \text{ Mpa}$); T1, Treatment 1 ($-0.40 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.60 \text{ Mpa}$); T2 Treatment 2 ($\phi_{\text{predawn}} \leq -0.6 \text{ Mpa}$).

3.5. Effects of Water Stress on the Transcriptional Level of Resveratrol Synthesis-Related Genes in Grape Berries

We then investigated the expression of the genes related to resveratrol biosynthesis for the treated berries (Figure 4). The results demonstrate that these genes are expressed differently for the same development stage. *VvPAL*, *Vv4CL*, *VvSTS*, *VvMYB14*, and *VvMYB15* were first expressed at 70 DAA. The expression levels increased rapidly and reached the

maximum at 90 DAA. Water stress causes upregulation of the genes. At 70 days after anthesis, the expression levels of *VvPAL*, *Vv4CL* and *VvSTS* in treated groups were higher than in the control. At 80 days after anthesis, the expression levels of *VvPAL*, *Vv4CL* and *VvMYB15* in T1 and T2 were higher than CK, while *VvSTS* and *VvMYB14* were lower than CK. The period of 90 days was like 70 days, in which the expression of PAL and STS was the largest in T1 and was significantly different from CK (2.4 and 2.6 times of CK). At 100 and 110 days after anthesis, the expression levels of *VvPAL*, *Vv4CL* and *VvSTS* in T1 and T2 were also higher than CK, and they were higher in T2 than in T1. In contrast, *VvMYB14* and *VvMYB15* were lower than CK and significantly different from CK. *VvCHS* shares the same substrate (4-coumaroyl CoA) with *VvSTS*. The expression of *VvCHS* in T1 and T2 was significantly lower than that of the control, indicating that water stress led to the downregulation of *VvCHS*.

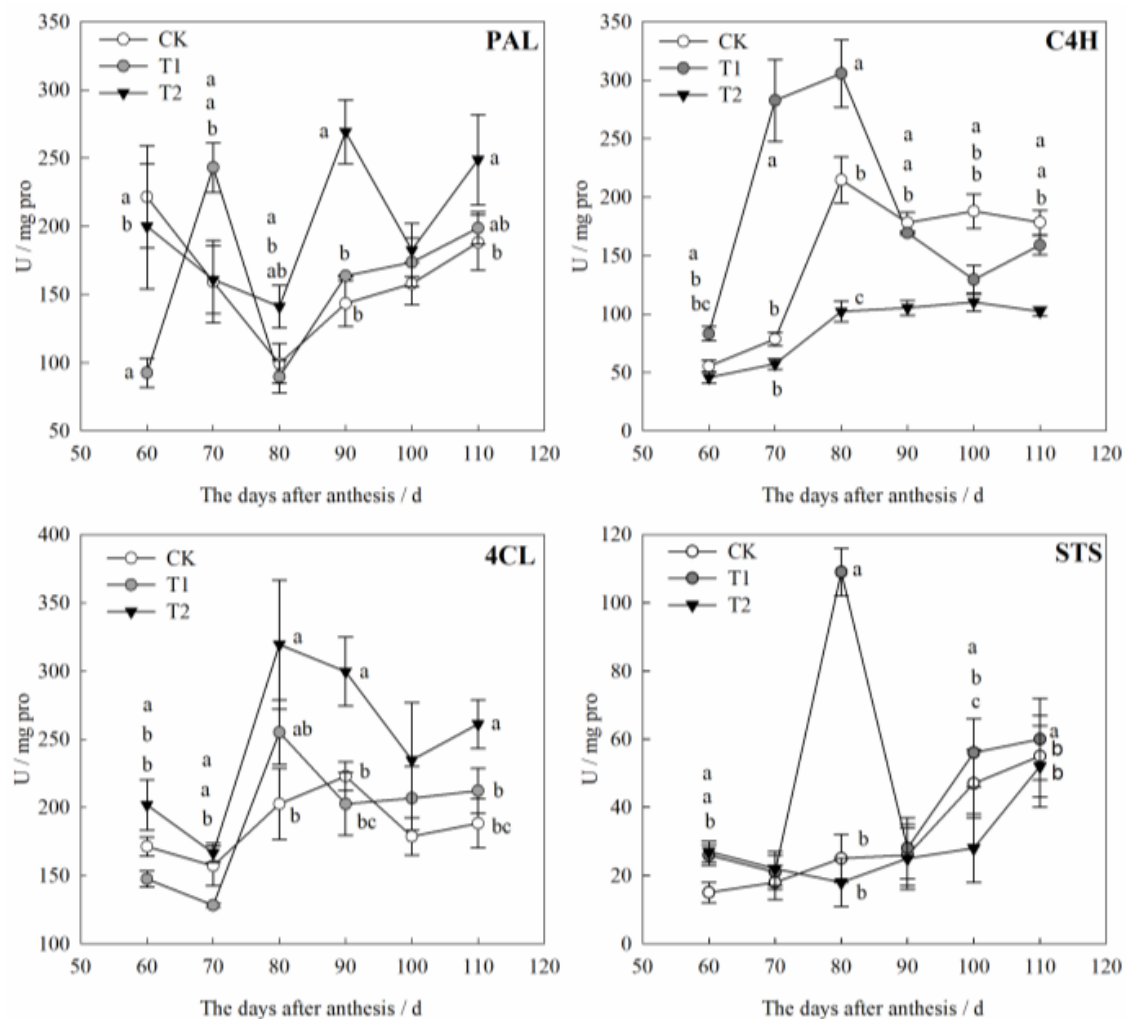


Figure 3. Effects of water stress on the activity of resveratrol-related synthases in grape berries. The value expressed as mean $\pm$ standard error (SD), values with different letters are significantly different according to Duncan's multiple range test (DMRT) at the 5% level. CK, Control ($-0.20 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.40 \text{ Mpa}$); T1, Treatment 1 ($-0.40 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.60 \text{ Mpa}$); T2 Treatment 2 ($\phi_{\text{predawn}} \leq -0.6 \text{ Mpa}$).

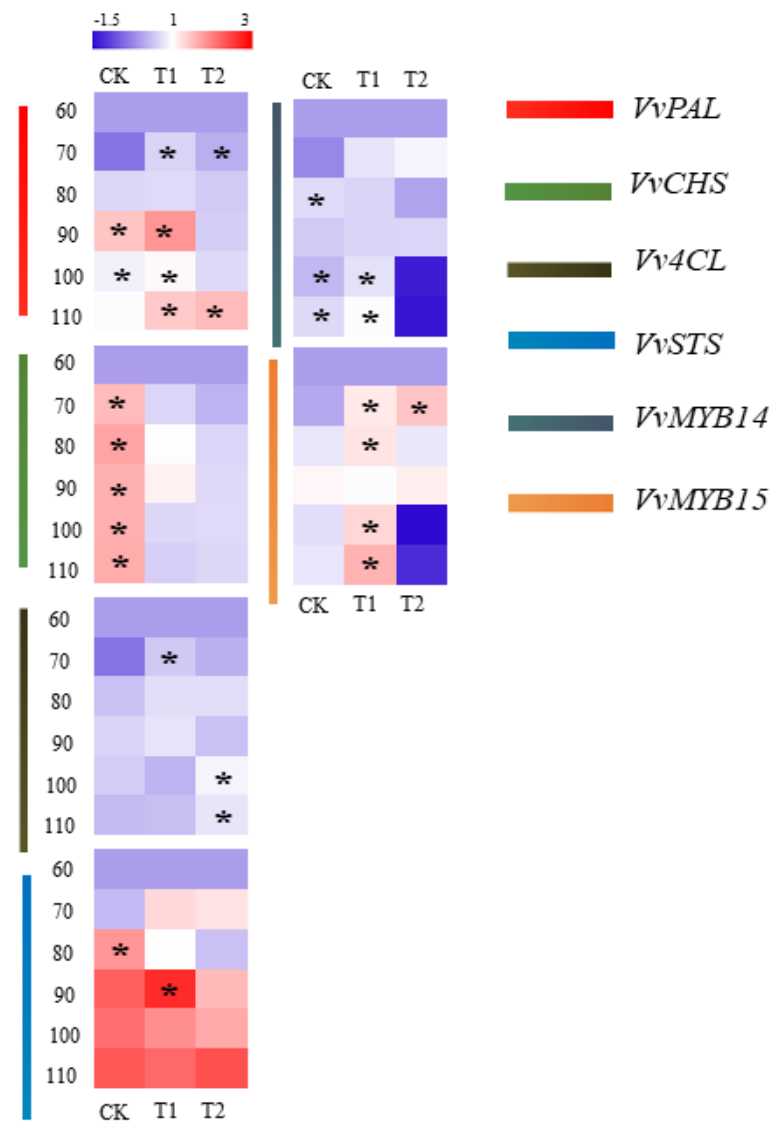


Figure 4. Effects of water stress on expression level of resveratrol synthesis-related genes and transcription factors (*VvPAL*, *Vv4CL*, *VvCHS*, *VvSTS*, *VvMYB14*, *VvMYB15*) in ‘cabernet sauvignon’. Heatmaps represent log₁₀ fold change (D/C) of the relative gene expression. Blue and red color shades indicate lower and higher relative gene expression. * Indicates significant differences ($p < 0.05$) between treatments. Different color patches correspond to different genes. CK, Control ($-0.20 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.40 \text{ Mpa}$); T1, Treatment 1 ($-0.40 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.60 \text{ Mpa}$); T2, Treatment 2 ($\phi_{\text{predawn}} \leq -0.6 \text{ Mpa}$).

3.6. Correlation Analysis between Transcriptional Level of Resveratrol Synthesis-Related Genes and Resveratrol Content

To gain a broader view of the genes involved in resveratrol biosynthetic pathways, the correlation between the transcriptional level of genes and resveratrol content was analyzed. During fruit ripening, there was a correlation between resveratrol and the transcription level of related genes (Table 2). The *VvSTS* gene in CK was significantly positively correlated with resveratrol content, and the correlation coefficient was 0.823 ($p < 0.05$). Water stress increases the transcription level of related genes. The *VvPAL* and *VvSTS* genes in T2 were significantly correlated with resveratrol content, and the correlation coefficients were 0.788 ($p < 0.05$) and 0.852 ($p < 0.05$), respectively.

Table 2. Correlation analysis of transcription level of resveratrol biosynthesis related genes and resveratrol content. * indicates significant differences at $p < 0.05$. CK, Control ($-0.20 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.40 \text{ Mpa}$); T1, Treatment 1 ($-0.40 \text{ Mpa} \geq \phi_{\text{predawn}} \geq -0.60 \text{ Mpa}$); T2 Treatment 2 ($\phi_{\text{predawn}} \leq -0.6 \text{ Mpa}$).

	PAL	4CL	STS	CHS	MYB14	MYB15
CK	0.240	0.415	0.823 *	0.671	0.589	0.335
T1	0.667	0.331	0.700	0.446	0.153	−0.033
T2	0.788 *	0.626	0.852 *	0.706	0.387	−0.440

4. Discussion

Water deficit strongly influenced berry metabolism, especially in the early stage of berry development. Water stress could accelerate the shift to anthocyanin biosynthesis and increase the initial rate of sugar accumulation. Moderate water stress affects the growth of primary and secondary shoots, and reduces the exposed leaf area, photosynthetic activity, berry growth, and the accumulation of sugar at the end of ripening [18]. In this study, three water treatments were applied to ‘Cabernet Sauvignon’ vines. The results showed that water stress negatively influenced the berry characteristics, decreased the sugar at maturity and accelerated the accumulation of anthocyanin and total polyphenols (Table 1). Some research shows that water stress on sugars and organic acids can be either positive or negative, depending on the type of degree of water deficit, and the period during which it is applied [23,24]. However, in contrast to the previous study, no effect on sugar contents was observed during the ripening process, revealing that excessive water stress will slow the synthesis and transportation of plant sugars. The decrease in titratable acid content may be due to the decrease in vigor of the plant resulting from water stress, which makes the berries easily exposed to sunlight, and the fruit temperature is increased. Berry size reductions increased the skin-to-pulp weight ratio and, consequently, the concentration of the different phenolic compounds within the berry skin. Therefore, the concentration of phenolic was always greater in the treated group berries than in CK.

It is well known that resveratrol is synthesized in response to biotic and abiotic stresses [11,25,26]. Our results indicate that the content of resveratrol and its derivatives increased with berry ripening. All the treatments enhanced the contents of *cis*- and *trans*-resveratrol, especially *trans*-resveratrol (Figure 2). Resveratrol content increased as the water stress increased, while the study in *Vitis. vinifera* cv. Barbera found that the accumulation of resveratrol did not increase under water stress [27]. The quantitative and qualitative composition of resveratrol depends on many factors including the cultivar, climatic conditions, developmental stage, agronomic management, storage conditions and postharvest treatments. Thus, the reason is probably due to resveratrol’s distribution in different cultivars or the differences in response to biotic or abiotic stress [28]. *Trans*- and *cis*-resveratrol can be transformed into *trans*- and *cis*-piceid by glycosyltransferases. In this study, T1 significantly increased *trans*-resveratrol while T2 mainly enhanced the *cis*-resveratrol. Both reduced the *cis*- and *trans*-piceid contents, suggesting that *Trans*- and *cis*-resveratrol are more sensitive to drought than the piceid form [29].

Different kinds of enzymes are involved in the biosynthesis pathway of stilbenes, PAL, 4CL, C4H and STS are essential enzymes involved in the phenylpropanoid pathway and related to the stilbenes. PAL is the crucial enzyme that catalyzes the formation of flavonoids and stilbenes. Water stress affects photosynthesis in plants and leads to the production of reactive oxygen species (ROS). When plants respond to severe water stress, phenolics can scavenge ROS during dehydration and release oxidative stress during recovery. Thus, enhanced activity of PAL was observed under severe water deficit conditions compared to controls [30]. Here, water stress increased the activity of the PAL enzyme, especially after 80 days. The PAL activity of T2 was higher than that of T1 and CK, which was similar to previous studies. The accumulation of resveratrol accompanied the increase in PAL enzyme activity. Correlation analysis showed that water stress increased the

correlation between PAL activity and resveratrol. The C4H enzyme is mainly involved in the lignification process of plants, and C4H activity can also be induced by chemical treatment and mechanical damage [31]. In this study, an increase in C4H activity occurred in the treatment group before veraison, indicating that the response of C4H to water stress was temporal and spatial. Similar to the C4H enzyme, the 4CL activity of the grape berries also increased first and then decreased. The STS enzyme is a crucial enzyme leading to the biosynthesis of resveratrol. Different resveratrol contents in plants probably depended on the catalytic ability of the STS enzyme [32]. We found that when plants were exposed to mild water stress (T1), the activity of STS increased significantly during the veraison stage. However, the increase in STS was not evident in the T2 group, revealing that the catalytic capacity of STS enzyme was different in responses to different water stresses and was related to berry development.

The activity of key enzymes is one of many factors that affects the accumulation of stilbenes in plants. Enzymes involved in the stilbene biosynthetic pathway were expressed following the expression of the corresponding genes. At the mRNA level, the PAL gene family comprising 17 genes in the grapevine expanded in parallel with the STS gene family in *Vitis* [33]. In this experiment, both showed similar gene expression trends. Water stress could increase the expression of the PAL gene and the accumulation of flavonoids. The gene was positively correlated with resveratrol content, indicating that water stress can increase resveratrol by inducing PAL [18]. Interactions between transcription factors and coordinate expression of different enzymes in the biosynthesis pathways have cooperative effects on stilbene's accumulation [34]. *VvMYB14* and *VvMYB15* were demonstrated to regulate STS gene expression [12]. The transcription levels of *VvMYB14*, *VvMYB15* and *VvSTS* in T2 were similar, while the correlation analysis showed that *VvMYB15* was negatively correlated with resveratrol. It is speculated that *VvMYB14* may participate in the regulation of *VvSTS* gene expression under water stress. STS and CHS compete for the same substrates, share very close amino acid sequences (which reaches 75–90% depending on the species), and possess very similar crystallographic structures [11]. The STS genes would be activated by stresses to synthesize resveratrol. In our study, water stress caused a significant decrease in the transcription level of CHS, while upregulating STS gene expression, indicating that the increase in resveratrol would help plants resist stresses.

The mechanism of accumulation of resveratrol in grapes is a complicated dynamic process. Hormones, transcription factors, and the related upstream/downstream genes regulate resveratrol synthesis. Because of disease resistance and the medicinal health functions of resveratrol, moderate water stress can be used to increase the content of resveratrol in grapes. Since the canopy structure and plant growth would be changed by water stress, the plants were exposed to sunlight, causing some target genes to be changed due to the light and thermal effects [35,36]. In addition, there are different types of elements in the upstream region of the STS gene. Therefore, we speculated that water stress regulated the synthesis of resveratrol through light-response elements in the upstream region of the STSs gene.

5. Conclusions

The analyses of berry quality and resveratrol carried out during this study demonstrate the potential effects of water supply on the biosynthesis of these substances during the development of the grapevine. Water stress increased the content of *cis*- and *trans*-resveratrol in mature fruits and decreased *cis*- and *trans*-piceid. In summary, water stress induced the accumulation of resveratrol in grape berries, mainly through increasing the activity of PAL and STS enzymes, up-regulating the expression of *VvPAL* and *VvSTS* genes, and reducing the expression of *VvCHS* in the post-veraison.

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Writing original draft, Y.S.; Reviewing and editing manuscript, Y.S. All authors have read and agreed to the published version of the manuscript.

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Appendix A

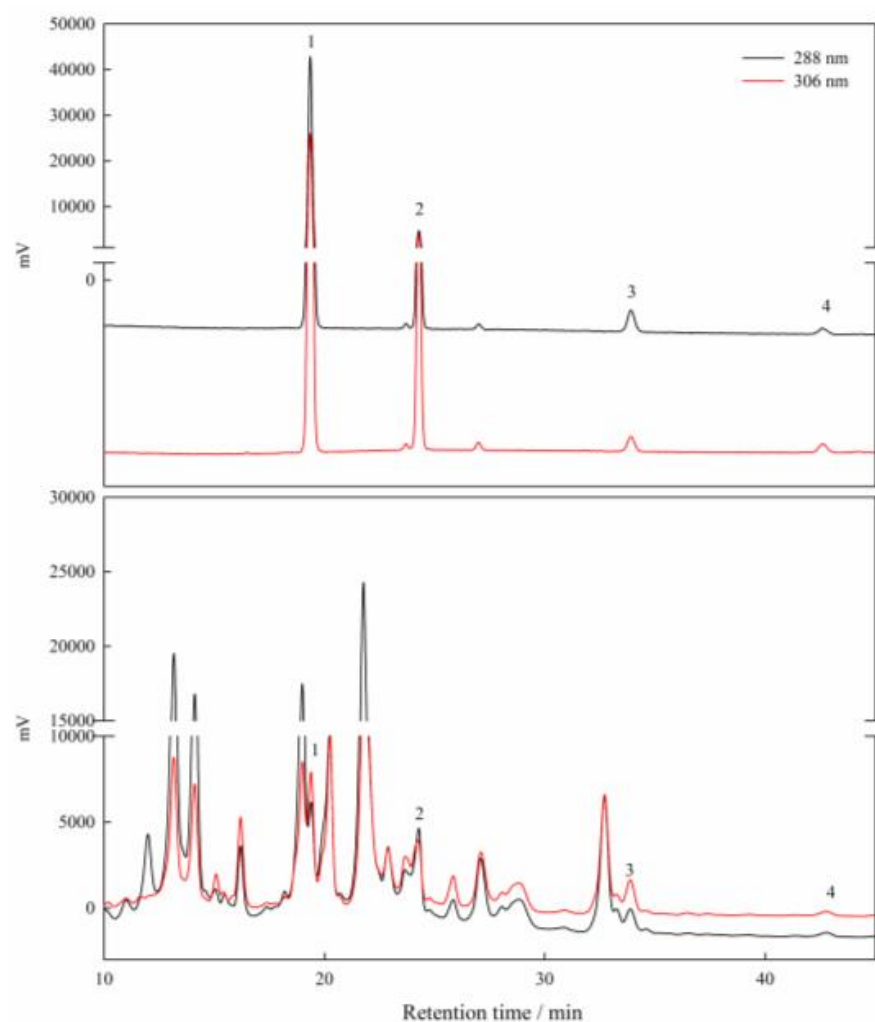


Figure A1. The HPLC chromatograms of standards of resveratrol and test sample. Numbers 1, 2, 3 and 4 are trans-piceid, cis-piceid, trans-resveratrol and cis-resveratrol, respectively.

Appendix B

Table A1. Primer sequences of real-time fluorescence quantitative PCR.

Gene Name	Primer Sequence (5'→3')		Accession No.
	Forward	Reverse	
PAL	AGTCATCCGAGCATCAACTAAA	CCACCATGTAGAGCCTTGTT	EF192469
4CL	GGAGAAGGTTTCACCGTCATTA	CGTCGGAGTTGATCGAAAC	JN858959
CHS	GCCAAGGCCATCAAAGAATG	TAGCAGCTTGGTGAGTTGATAG	AF020709
STS	CAGCAGCCCAAACATTTATTCC	GTTCCAATCGCTAATACCAAGTG	KX523623
Myb14	TCTGAGGCCGGATATCAAAC	GGGACGCATCAAGAGAGTGT	EU181424
Myb15	CAAGAATGAACAGATGGAGGAG	TCTGCGACTGCTGGGAAA	KX523624
EF1	GTAAAGATGATTCCAACCAAGCCC	TCTCCACGCTCTTGATGACTC	XM_002279562
Actin	TCCTTGCCTTGCCTCATCTAT	CACCAATCACTCTCTGCTACAA	XM_002277287

References

- Cantos, E.; Espín, J.C.; Tomás-Barberán, F.A. Postharvest stilbene-enrichment of red and white table grape varieties using UV-C irradiation pulses. *J. Agric. Food Chem.* **2002**, *50*, 6322–6329. [\[CrossRef\]](#)
- Li, H.G.; Xia, N.; Forstmann, U. Cardiovascular effects and molecular targets of resveratrol. *Nitric Oxide - Biol. Chem.* **2012**, *26*, 102–110. [\[CrossRef\]](#)
- Akabeti, M.; Hosseinzadeh, H. Grapes (*Vitis vinifera*) as a Potential Candidate for the Therapy of the Metabolic Syndrome. *Phytother. Res.* **2016**, *30*, 540–556. [\[CrossRef\]](#)
- Nassiri-Asl, M.; Hosseinzadeh, H. Review of the Pharmacological Effects of *Vitis vinifera* (Grape) and its Bioactive Constituents: An Update. *Phytother. Res.* **2016**, *30*, 1392–1403. [\[CrossRef\]](#)
- Delmas, D. Transport, stability, and biological activity of resveratrol. *Resveratrol. Health* **2011**, *1215*, 48–59. [\[CrossRef\]](#)
- Figueiras, T.S.; Neves-Petersen, M.T.; Petersen, S.B. Activation Energy of Light Induced Isomerization of Resveratrol. *J. Fluoresc.* **2011**, *21*, 1897–1906. [\[CrossRef\]](#)
- Soudi, I. The effect of salt stress on resveratrol and piceid accumulation in two *Vitis vinifera* L. cultivars. *Physiol. Mol. Biol. Plants* **2019**, *25*, 625–635. [\[CrossRef\]](#)
- Averilla, J.N. Improved extraction of resveratrol and antioxidants from grape peel using heat and enzymatic treatments. *J. Sci. Food Agric.* **2019**, *99*, 4043–4053. [\[CrossRef\]](#)
- Parage, C. Structural, Functional, and Evolutionary Analysis of the Unusually Large Stilbene Synthase Gene Family in Grapevine. *Plant Physiol.* **2012**, *160*, 1407–1419. [\[CrossRef\]](#)
- Valletta, A.; Iozia, L.M.; Leonelli, F. Impact of Environmental Factors on Stilbene Biosynthesis. *Plants* **2021**, *10*, 90. [\[CrossRef\]](#)
- Vannozzi, A. Genome-wide analysis of the grapevine stilbene synthase multigenic family: Genomic organization and expression profiles upon biotic and abiotic stresses. *BMC Plant Biol.* **2012**, *12*, 1–22. [\[CrossRef\]](#) [\[PubMed\]](#)
- Holl, J. The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis in *Vitis vinifera*. *Plant Cell* **2013**, *25*, 4135–4149. [\[CrossRef\]](#) [\[PubMed\]](#)
- Gatto, P. Ripening and Genotype Control Stilbene Accumulation in Healthy Grapes. *J. Agric. Food Chem.* **2008**, *56*, 11773–11785. [\[CrossRef\]](#) [\[PubMed\]](#)
- Dimopoulos, N. Drought stress modulates cuticular wax composition of the grape berry. *J. Exp. Bot.* **2020**, *71*, 3126–3141. [\[CrossRef\]](#)
- Pannu, N.; Bhatnagar, A. Resveratrol: From enhanced biosynthesis and bioavailability to multitargeting chronic diseases. *Biomed. Pharm.* **2019**, *109*, 2237–2251. [\[CrossRef\]](#) [\[PubMed\]](#)
- Oraee, A.; Tehranifar, A. Evaluating the potential drought tolerance of pansy through its physiological and biochemical responses to drought and recovery periods. *Sci. Hortic.* **2020**, *265*, 109225. [\[CrossRef\]](#)
- Bahar, E. The effect of extreme water stress on leaf drying limits and possibilities of recovering in three grapevine (*Vitis vinifera* L.) cultivars. *Afr. J. Agric. Res.* **2011**, *6*, 1151–1160.
- Castellarin, S.D. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* **2007**, *227*, 101–112. [\[CrossRef\]](#)
- Lamb, C.J.; Rubery, P.H. A spectrophotometric assay for trans-cinnamic acid 4-hydroxylase activity. *Anal. Biochem.* **1975**, *68*, 554–561. [\[CrossRef\]](#)
- Knobloch, K.H.; Hahlbrock, K. Isoenzymes of p-coumarate: CoA ligase from cell suspension cultures of *Glycine max*. *Eur. J. Biochem.* **1975**, *52*, 311–320. [\[CrossRef\]](#)
- Lister, C.E. Developmental changes in enzymes of flavonoid biosynthesis in the skins of red and green apple cultivars. *J. Sci. Food Agric.* **1996**, *71*, 313–320. [\[CrossRef\]](#)
- Krzyzaniak, Y. Combined enzymatic and metabolic analysis of grapevine cell responses to elicitors. *Plant Physiol. Biochem.* **2018**, *123*, 141–148. [\[CrossRef\]](#)

23. Ojeda, H. Influence of pre-and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002**, *53*, 261–267.
24. Wang, Z.P.; Deloire, A.; Carbonneau, A.; Federspiel, B.; Lopez, F. An in vivo experimental system to study sugar phloem unloading in ripening grape berries during water deficiency stress. *Ann. Bot.* **2003**, *92*, 523–528. [[CrossRef](#)]
25. Versari, A. Stilbene Compounds and Stilbene Synthase Expression during Ripening, Wilting, and UV Treatment in Grape cv. Corvina. *J. Agric. Food Chem.* **2001**, *49*, 5531–5536. [[CrossRef](#)]
26. Borie, B. Resveratrol and stilbene synthase mRNA production in grapevine leaves treated with biotic and abiotic phytoalexin elicitors. *Am. J. Enol. Vitic.* **2004**, *55*, 60–64. [[CrossRef](#)]
27. Vezzulli, S. Methyl jasmonate treatment as a trigger of resveratrol synthesis in cultivated grapevine. *Am. J. Enol. Vitic.* **2007**, *58*, 530–533. [[CrossRef](#)]
28. Wang, L. Resveratrols in grape berry skins and leaves in *Vitis* germplasm. *PLoS ONE* **2013**, *8*, e61642. [[CrossRef](#)]
29. Villangó, S. The effect of postveraison water deficit on the phenolic composition and concentration of the Kékfrankos (*Vitis vinifera* L.) berry. *Sci. Hortic.* **2016**, *209*, 113–116. [[CrossRef](#)]
30. Hura, T.; Hura, K.; Grzesiak, S. Contents of total phenolics and ferulic acid, and PAL activity during water potential changes in leaves of maize single-cross hybrids of different drought tolerance. *J. Agron. Crop Sci.* **2008**, *194*, 104–112. [[CrossRef](#)]
31. Huntley, S.K. Significant increases in pulping efficiency in C4H-F5H-transformed poplars: Improved chemical savings and reduced environmental toxins. *J. Agric. Food Chem.* **2003**, *51*, 6178–6183. [[CrossRef](#)]
32. Guo, H. Comparison of stilbene synthase from different plant sources for resveratrol biosynthesis. *Sheng Wu Gong Cheng Xue Bao Chin. J. Biotechnol.* **2014**, *30*, 1622–1633.
33. Chong, J.; Poutaraud, A.; Hugueney, P. Metabolism and roles of stilbenes in plants. *Plant Sci.* **2009**, *177*, 143–155. [[CrossRef](#)]
34. Lu, D.; Zhao, W.; Zhao, S. Relevant Enzymes, Genes and Regulation Mechanisms in Biosynthesis Pathway of Stilbenes. *Open J. Med. Chem.* **2012**, *2*, 15–23.
35. Chaves, M.M. Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Ann. Appl. Biol.* **2007**, *150*, 237–252. [[CrossRef](#)]
36. Castellarin, S.D. Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell* **2007**, *30*, 1381–1399. [[CrossRef](#)]

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