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Individual and Combined Effects of $CaCl_2$ and UV−C on the Biosynthesis of Resveratrols in Grape Leaves and Berry Skins

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ABSTRACT: The individual and combined effects of calcium chloride ($CaCl_2$) and ultraviolet C (UV−C) light on the synthesis of resveratrol in grape leaves and berry skins were investigated. Results showed that all treatments could increase leaf resveratrol contents at least about 5 times, but the combination treatment was the most efficient. Moreover, compared with UV−C treatment, the combination treatment delayed the decline of resveratrol contents. The expression levels of phenylalanine ammonia lyase (*PAL*), cinnamate-4-hydroxylase (*C4H*), coumaroyl-CoA ligase (*4CL*), and stilbene synthase (*STS*) and 3-*O*-*β*-glycosyltransferases, which are related to the synthesis of resveratrol, increased in response to these treatments, paralleling the change in resveratrol content. All treatments also induced the biosynthesis of resveratrol in berry skins at room temperature. The berries of these treatments held at room temperature for 1 day were further stored under low temperature (−1 ± 0.5 °C, RH 95%) for 27 days, and the results showed that all treatments continuously increased berry skin resveratrol content, with the combination treatment being most efficient. During cold storage, resveratrol content remained at high levels and reached a maximum (about 247.7 μg/g FW) at 13 days, then showed a slight decline, though it remained high by the end of storage. Berry firmness and total soluble solids content showed slight changes during cold storage, but there were no differences among the treatments. Thus, the combination treatment of $CaCl_2$ and UV−C could be an efficient method for increasing resveratrol content of table grapes during storage under low temperature. This would be potentially beneficial for producing functional fruits.

KEYWORDS: *grape, resveratrol, UV−C, $CaCl_2$*

INTRODUCTION

Resveratrol (3,4′,5-trihydroxystilbene, Res), is a secondary metabolite which is produced by plants as a self-defense agent. In recent years, more and more attention has been paid to Res due to its positive health effects: inhibiting tumors and low density lipoprotein oxidation,[1] and for preventing cardiovascular disease[2] and cancer.[3,4] It has only been found in some plants such as grapevine,[5,6] pine,[7] and peanut.[8] Grapes and grape products are the most important potential sources from which people obtain Res. Res exists as *trans*- or *cis*-resveratrol (monomers) but also as piceids (glucoside derivatives). Res is found in the berry, stem, axillary bud, shoot tip, petiole, root, and leaf of grapevines.[9] However, Res content varies with grape organs, and depends on genotype. Its biosynthesis and accumulation in grape tissues under natural conditions is usually low, especially in *V. vinifera* table grapes.[10]

Res is formed by the phenylalanine pathway, through phenylalanine ammonia lyase (PAL) (EC 4.3.1.5), cinnamate-4-hydroxylase (C4H) (EC 1.14.13.11), coumaroyl-CoA ligase (4CL) (EC 6.2.1.12), and stilbene synthase (STS) (EC 2.3.1.95). STS directly produces *trans*- resveratrol (*Trans*-res) which may isomerize to *cis*- resveratrol (*cis*-res). These Res may also be glucosylated into *trans*-piceid (*trans*-pd) and *cis*-piceid (*cis*-pd) by 3-*O*-*β*-glycosyltransferases (*O*-3-*GT*).[11]

Biosynthesis of Res in grape berries and other organs such as leaves can be stimulated by a number of biotic and abiotic factors.[12] Ultraviolent C (UV−C) exposure has long been known as an efficient inducer of Res biosynthesis in grapevine.[5,13−19] STS is very sensitive to UV−C; however, there is almost no natural UV−C in sunlight at the earth's surface.[13] This is one reason for the low level of Res in most berries and leaves under natural conditions. In general, Res content in grape berries and leaves reached maximum at 24−48 h after UV−C treatment at room temperature and in the dark, then rapidly declined.[5,13−17,20] This may be because of poor application of UV−C in practice. Another factor is Ca^{2+}, which is a crucial regulator of growth and development in plants. The myriad processes in which this ion participates are involved in nearly all aspects of plant development.[21] Ca^{2+} may function to maintain high Res content in leaves or berries treated by UV−C.

Grapes store poorly at room temperature and are thus usually stored under low temperature. Prestorage treatment of table grapes, tomato, mango, and citrus fruit with low doses of UV−C was reported to reduce postharvest decay of fruit.[22−24] In addition, many studies indicate that calcium chloride ($CaCl_2$) plays an important role in affecting the quality and preservation of fruits and vegetables. Calcium has been utilized to maintain quality, prevent softening, reduce the rate of rot,

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and prolong shelf life for harvested fruits and vegetables, including peach, grape, strawberry, pear, and sweet pepper.[25−29] Pre- and postharvest application of calcium may help to reduce senescence during commercial and retail storage of fruit, with no detrimental effect on consumer acceptance.[30] However, the effect of $CaCl_2$ on Res biosynthesis before or during berry storage is unknown.

Grape leaves also have a relatively remarkable content of Res.[9,17] However, in the past, less attention has been paid to the biosynthesis of Res in leaves as influenced by abiotic and biotic factors. Actually, if the biosynthesis of Res in leaves is inducible, it should be possible to exploit another resource for Res industry. The present study was designed to investigate the effects of UV−C and $CaCl_2$, individually and combined, on the biosynthesis of Res in grape leaves and skins at normal temperature. Moreover, Res content was also investigated in treated berry skins stored at low temperature.

MATERIALS AND METHODS

Plant Material and Treatments. Two cultivars, 'Beihong' (*Vitis vinifera* × *V. amurensis*) and 'Hongbaladuo' (*V. vinifera*) were used in leaf and berry experiments, respectively, in this study. All grapevines of the two cultivars were grown in the experimental vineyard at the Institute of Botany, Chinese Academy of Sciences, Beijing, China.

Leaf experiment: Mature (30 day) and healthy leaves of 'Beihong' with similar size were used. Three individual subexperiments were designed. (1) $CaCl_2$ treatments: leaves were divided into three groups and dipped into $CaCl_2$ solutions of three different concentrations (5, 15, and 50 mM) for 2 h, and the petioles were then incubated in triangular flasks containing $CaCl_2$ solutions (5, 15, and 50 mM) until sampling. The most effective concentration of $CaCl_2$ solutions for the synthesis of Res was chosen according to the results. (2) UV−C treatment: leaves (abaxial sides) were irradiated with UV−C (254 nm, Spectroline, Model ZQJ-254, power 6 W/m^2) at 15 cm distance for 10 min, and the leaf petioles were then inserted into triangular flasks containing ddH_2O till sampling. (3) Combination treatment of $CaCl_2$ and UV−C: after immersing the leaves into a $CaCl_2$ solution (50 mM) for 2 h, leaves were irradiated with UV−C (6 W/m^2) for 10 min. Petioles were inserted into triangular flasks containing $CaCl_2$ solutions (50 mM) throughout the treatment till sampling. The control leaves were neither subjected to $CaCl_2$ application nor UV−C irradiation, but the leaf petioles were incubated in triangular flasks containing only H_2O until sampling. All of the leaves subjected to $CaCl_2$, UV−C, or $CaCl_2$ and UV−C combination treatments and control leaves were incubated in the dark at 25 °C for 48 h (including treatment time). Samples were collected at 0, 12, 24, and 48 h after treatment. All of the treatments had three independent replicates, and each replicate consisted of 6 leaves. After sampling, the leaves were ground into powder in liquid nitrogen and then stored at −80 °C until analysis.

Berry experiment: 'Hongbaladuo' grape berries during ripening were used to conduct three different treatments. (1) $CaCl_2$ treatment: berries were dipped into a $CaCl_2$ solution (50 mM) for 2 h. (2) UV−C treatment: berries were irradiated with UV−C (the same as that described above) at 15 cm distance for 10 min. At half of the irradiated time, berries were turned over to make sure they were irradiated equally on both sides. (3) The combined treatment of $CaCl_2$ and UV−C: berries were irradiated with UV−C (the same as that described above) at 15 cm distance for 10 min after dipping into the $CaCl_2$ solution (50 mM) for 2 h. Control: berries without any treatments. All of the treatments and controls were incubated in the dark at room temperature (25 °C) for 24 h (including treatment time). Subsamples of berries of all treatments were sampled. The remaining berries were then stored in a cold environment (−1 ± 0.5 °C, RH 95%) for 27 days. Samples were collected at 6, 13, 20, and 27 days. Each treatment had three independent replicates, and each replicate consisted of 50 berries. At sampling, fruit firmness and total soluble solids (TTS) were immediately assessed. Flesh firmness was quantified by a puncture test using a digital penetrometer (Digital Fruit Firmness Tester, TR Turoni S.r.l., Forlì, Italy) fitted with a 3 mm diameter plunger. After skin removal, the plunger was inserted, at the berry equator, on opposite sides, to a depth of 7 mm. Total soluble solids (TSS) were measured using a digital refractometer in juice obtained by squeezing, homogenizing, and filtering peeled berries. The berry skins were peeled, ground into powder in liquid nitrogen, and then stored at −80 °C until analysis.

Resveratrol Determination. The Res in leaves and berry skins was extracted according to the methods described by Liu et al.[31] Briefly, 1 g of berry skins or leaves was extracted with 10 mL of methanol/ethyl acetate (1:1, v/v) for 24 h at room temperature under darkness. After centrifugation at 10,000*g* and 4 °C for 10 min, the supernatant was evaporated at 40 °C to dryness and then dissolved in 2 mL of methanol. The extract was filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane before high-performance liquid chromatography (HPLC) analysis.

All samples were analyzed using a Dionex P680 HPLC system (Dionex Corporation, CA, USA) equipped with a reverse-phase C18 column of Atlantis T3 (5-μm particle sizes, 4.6 mm × 250 mm I.D.; Waters, USA) and a C18 Nova Pack guard precolumn (Waters). Injection volume was 10 μL, and column temperature was 30 °C. *cis*-Isomers were detected at 288 nm and *trans*-isomers at 306 nm, and photodiode array spectra were recorded from 240 to 600 nm. Separation was performed at a flow rate of 1.0 mL/min with the mobile phase consisting of acetonitrile (A) and ddH_2O (B). The solvent gradient was as follows: 0 min, 10% solvent A; 5 min, 17% A; 12 min, 18% A; 22 min, 22% A; 30 min, 33% A; 45 min, 38% A; and 58 min, 100% A. The fluorimetric detection for *cis*-isomers was at 288 nm, while that for *trans*-isomers was at 306 nm. The maximum excitation wavelength was measured at 240 nm and emission at 600 nm. In all samples, we detected *trans*-res, *trans*-pd, and *cis*-pd except for *cis*-res. The total Res content indicates the sum of *trans*-res, *trans*-pd, and *cis*-pd contents.

Preparation of Total RNA and cDNA and RT-PCR Analysis. The expression of *PAL*, *C4H*, *4CL*, *STS*, and *O-3-GT* genes were analyzed in leaf samples. Total RNA was isolated using Plant Total RNA Extraction Kit (Bioteke, Beijing, China) according to the manufacturer's protocol. cDNA of grape leaves was prepared using the reagents and methods according to the manufacturer's protocol (Promega, Beijing, China). The primers in Table 1 were designed according to the grape genome. A PCR program was operated in a Rotor-Gene 3000 Amp PCR system (Agilent Technologies) as follows: 1 μL of cDNA, 0.6 μL of each gene-specific amplification primer, and 8.8 μL of ultrapure water were prepared. Predenaturation was at 94 °C for 2 min; 40 cycles of amplification (denaturation at 94 °C for 10 s, annealing at 55 °C for 18 s, and extension at 68 °C for 20 s) were conducted, with a final extension at 72 °C for 10 min. Fluorescence signals were captured at the end of each cycle, and a melting curve analysis was performed from 68 to 95 °C. The amplification of actin rRNA gene sequence was used as the internal

Table 1. Primers of the Genes Related to Resveratrol Synthesis

gene name	primers
Actin	forward: 5′-CTTGCATCCCTCAGCACCTT-3′
	reverse: 5′-TCCTGTGGACAATGGATGGA-3′
PAL	forward: 5′-CAACCAAGATGTGAACTCCTT-3′
	reverse: 5′-TTCTCCTCCAAATGCCTC-3′
C4H	forward: 5′-GGCAAGCACAAAGAGCACAGAT-3′
	reverse: 5′-TTCTTCTGGATGTGAGGGTGGTT-3′
4CL	forward: 5′-CGAAGAACCCGATGGTGGAGA-3′
	reverse: 5′-CACGAGCCGGACTTAGTAGGA-3′
STS	forward: 5′-TAGAAACGCTCAACGTGCCAAGGG-3′
	reverse: 5′-ATCAGCATAATCAGACTGGTAGAC-3′
O-3-GT	forward: 5′-GGCTTCAAAGGGCTTGCTTGTG-3′
	reverse: 5′-GGCGTTGGTTGGTCAGTGATGT-3′

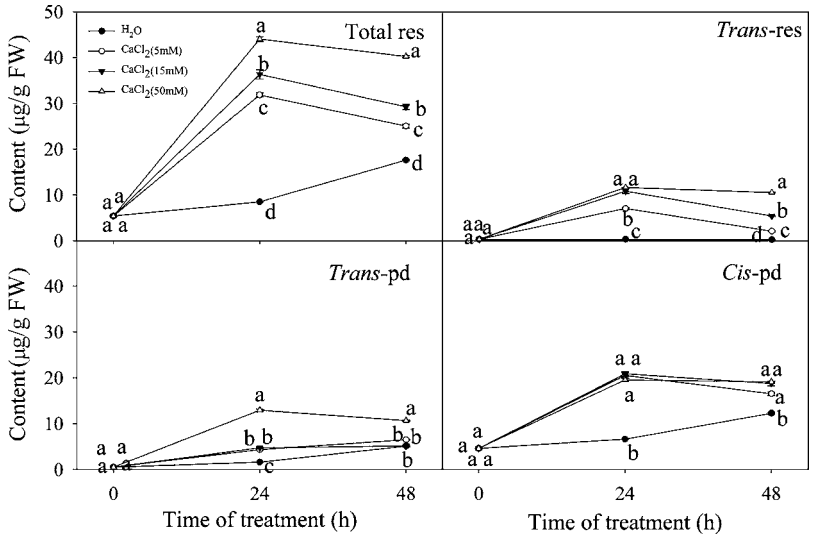

Figure 1. Effect of $CaCl_2$ solution of different concentrations on resveratrol contents in leaves of 'Beihong'. Mature and healthy leaves were divided into three groups and dipped into $CaCl_2$ solutions of three different concentrations (5, 15, and 50 mM) for 2 h, respectively. The petioles were then incubated in triangular flasks containing the corresponding $CaCl_2$ solutions until sampling. The means of three replicates and their SE are presented. Different letters indicate significant differences among the treatments at $P < 0.05$.

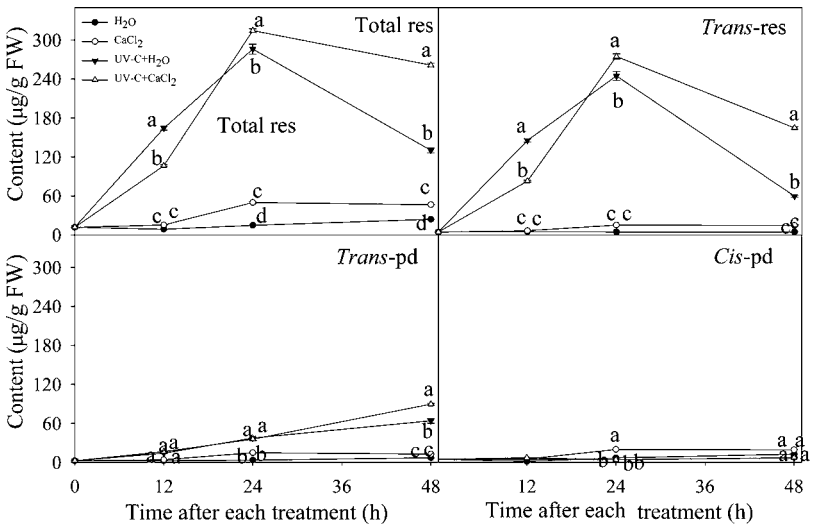

Figure 2. Effect of UV−C, $CaCl_2$ (50 mM), and combined treatments on resveratrol contents in leaves of 'Beihong'. Mature and healthy leaves of 'Beihong' were used. $CaCl_2$ treatment: the treatment method is the same as that described in Figure 1 with 50 mM $CaCl_2$ solution. UV−C + H_2O treatment: after leaves (abaxial sides) were irradiated with UV−C for 10 min, the leaf petioles were inserted into triangular flasks containing ddH_2O until sampling. UV−C + $CaCl_2$ treatment: after leaves (abaxial sides) were irradiated with UV−C for 10 min, the leaf petioles were inserted into triangular flasks containing 50 mM $CaCl_2$ solutions until sampling. The control (H_2O): leaves were neither subjected to $CaCl_2$ application nor UV−C irradiation. Leaf petioles were incubated in triangular flasks containing only H_2O until sampling. The means of three replicates and their SE are presented. Different letters indicate significant differences among the treatments at $P < 0.05$.

control to normalize all of the data.[32,33] Analyses of qRT-PCR data used the classic $(1 + E)^{-\Delta\Delta CT}$ method (C_T is the threshold cycles of one gene, and E is the amplification efficiency). ΔC_T is equal to the difference in threshold cycles for the target (X) and reference (R) ($C_{T,X} - C_{T,R}$), while $\Delta\Delta C_T$ is equal to the difference of ΔC_T for the control (C) and treatment (T) groups ($\Delta C_{T,T} - \Delta C_{T,C}$). The amplification system (e.g., primer and template concentrations) was properly optimized, and the efficiency was close to 1. Therefore, the amount of target, normalized to an endogenous reference and relative to a calibrator, is given by the following equation:

$$\text{amount of target} = 2^{-\Delta\Delta CT}$$

Graphs and Data Analysis. Graphs of the experimental data were developed using Sigma Plot 10.0 (SPSS Inc., Chicago, USA) for Windows. The concentration of each compound was plotted over sampling periods from three replicates, and experimental data were subjected to analysis of variance using PASW Statistics 18.0 (SPSS Inc.). Means were separated by Student−Newman−Keuls's multiple range tests at $P < 0.05$.

RESULTS

Res Contents in Grape Leaves Induced by $CaCl_2$ at Different Concentrations. All of the $CaCl_2$ applications significantly stimulated the accumulation of *trans*-res, *trans*-pd, and *cis*-pd and the total Res in leaves compared with those in the control (Figure 1). Moreover, the accumulation of Res depended on applied $CaCl_2$ concentration. Total Res increased in the leaves treated with increased $CaCl_2$ concentrations. Total Res content in leaves reached a maximum at 24 h after

supplying any $CaCl_2$ treatment. At this time, total resveratrol contents in leaves treated with $CaCl_2$ solutions (5, 15, and 50 mM) were 25.0, 29.2, and 40.2 μg/g FW, which were 1.4, 1.7, and 2.3 times the control content, respectively. At 48 h, total Res in all leaves treated with these $CaCl_2$ solutions decreased to 25.0, 29.2, 40.2 μg/g FW. For all of the $CaCl_2$ treatments, the changes in *trans*-res had a tendency similar to those of total Res content. With regard to *trans*-pd, 50 mM $CaCl_2$ resulted in higher content than 5 and 15 mM $CaCl_2$ treatments at 48 h, and the effect of 5 and 15 mM $CaCl_2$ treatments on *trans*-pd disappeared at 48 h after $CaCl_2$ application. However, all $CaCl_2$ applications resulted in higher *cis*-pd content than that of the control, and there were no differences among the treatments. All of the results show that a 50 mM $CaCl_2$ solution was the most efficient concentration to induce Res synthesis, and it was selected for further studies.

Res Content in Grape Leaves under UV−C, $CaCl_2$, and Combination Treatments. *trans*-res, *trans*-pd, and *cis*-pd and total Res contents changed little in control leaves incubated in H_2O throughout the experimental periods (Figure 2). Compared with the control, the total Res in grape leaves significantly increased in individual $CaCl_2$, UV−C, and combination treatments, reaching peak levels at 24 h during incubation, then declined although they remained higher than those of the controls. At 24 h of treatment, resveratrol contents in leaves of $CaCl_2$, UV−C, and the combination treatments were 44.0, 286.1, and 314.6 μg/g FW, which were 5.2, 33.8, and 37.2 times the control level, respectively. At the end of the experiment, total resveratrol in the combination treatment was significantly higher than that of the UV−C treatment. The trends of *trans*-res content in the different treatments were similar to those of total resveratrol, and *trans*-res was the main component of total resveratrol. *trans*-pd levels of the combination and UV−C treatments increased continuously during the experiment, but they were not significantly different before 24 h. At 48 h, the content of the combination treatment was higher than that of the UV−C treatment. With regard to *cis*-pd, the content in different treatments was very low, and there were no treatment effects (Figure 2).

Res in the Berry Skins Induced by UV−C and $CaCl_2$ at Room Temperature (25 °C). Berries treated by $CaCl_2$, UV−C, and a combination of the two were held at room temperature (25 °C) in the dark for 24 h, as were the control berries. Similar to leaves, *cis*-res was not detected in berry skins (Table 2). The 50 mM $CaCl_2$ application had no effect on *trans*-pd, *cis*-pd, and total Res contents, but it resulted in significantly higher content in *trans*-res compared with that in the control. UV−C and combination treatments increased Res synthesis and accumulation in berry skins. The *trans*-res, *trans*-pd, *cis*-pd, and total Res contents in the UV−C treatment skins were 67.6, 7.7, 1.4, and 76.7 μg/g FW, respectively, and 103.4, 7.1, 0.9, and 111.5 μg/g FW, respectively, in combined treatment skins.

Table 2. Total Res Contents ($\mu g \cdot g^{-1}$ FW) in the Berry Skins in the Dark at the Room Temperature (25°C) 24 h after UV−C, $CaCl_2$, and Their Combined Treatments[a]

treatments	*trans*-res	*trans*-pd	*cis*-res	*cis*-pd	total Res
control	6.20 d	4.33 b	0	2.24 a	12.77 c
$CaCl_2$	9.38 c	4.53 b	0	1.87 ab	15.78 c
UV−C	67.60 b	7.72 a	0	1.37 bc	76.69 b
UV−C + $CaCl_2$	103.43 a	7.12 a	0	0.94 c	111.49 a

[a]Different letters indicate significant differences between the treatments at $P < 0.05$. The means of three replicates are presented.

Res in the Berry Skins, Berry Firmness, and TTS during Low Temperature Storage. As shown in Figure 3,

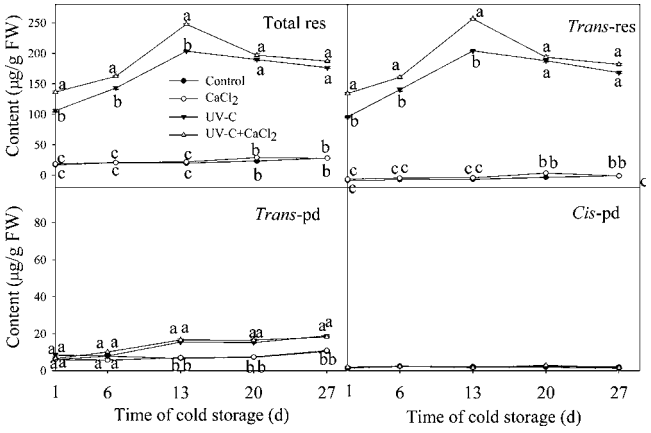

Figure 3. Changes of resveratrol contents in berries during storage (−1 ± 0.5 °C) after UV−C, $CaCl_2$ (50 mM), and the combined treatments. $CaCl_2$ treatment: berries were dipped into a $CaCl_2$ solution (50 mM) for 2 h. UV−C treatment: berries were irradiated with UV−C for 10 min. UV−C + $CaCl_2$ treatment: berries were irradiated with UV−C for 10 min after dipping into the $CaCl_2$ solution (50 mM) for 2 h. The control: berries without any treatments before storage. Each treatment had three independent replicates, and each replicate consisted of 50 berries. The means of three replicates and their SE are presented. Different letters indicate significant differences among the treatments at $P < 0.05$.

UV−C and UV−C + $CaCl_2$ treatments continued to accumulate *trans*-res, *trans*-pd, and total Res in berry skin until two weeks after cold storage. Then, *trans*-res and total resveratrol contents decreased to some extent during the rest of storage, while *trans*-pd increased continuously until the end of storage. *cis*-pd content was very low for all of the treatments and the control, and there were no differences in *cis*-pd content among treatments. During the storage of grape berries, total resveratrol content in the control and $CaCl_2$ treatments slowly increased, and there was no difference in total Res among them.

During cold storage, berry firmness declined slightly (approximately 2.5−2.3 kg/cm^2), while TTS increased slightly (approximately 13.3−14.5%). However, there was no difference in these parameters among treatments and the control (Figure 4).

Expression of *PAL*, *C4H*, *4CL*, *STS*, and *O-3-GT* in Leaves under UV−C, $CaCl_2$ and Combination Treatments. As shown in Figure 5, UV−C and combined treatments significantly stimulated the expression of *PAL*, *C4H*, *4CL*, *STS*, and *O-3-GT*, which were related to the synthesis of Res. Moreover, the expression levels of these genes in the UV−C and $CaCl_2$ combination treatment were higher than those of the UV−C treatment. The expression of *PAL*, *C4H*, *4CL*, and *STS* in both treatments reached a peak at 12 h after initiating the treatment, then declined rapidly and was close to the control level at the end of the experiment. However, the expression of *O-3-GT* reached a maximal level at 24 h, then decreased quickly and also approached the control level. $CaCl_2$ treatment alone did not significantly modify the expression of any of these genes.

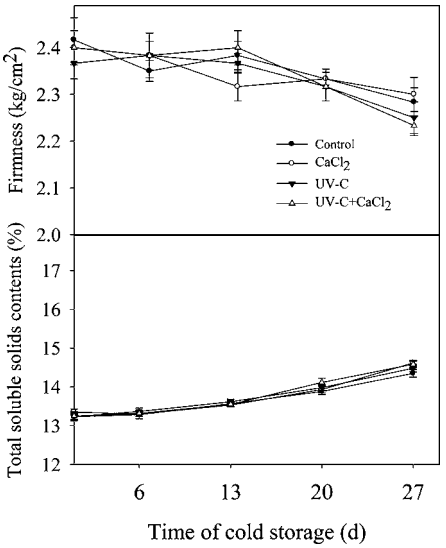

Figure 4. Changes of berry firmness and total soluble solids in berries during storage (−1 ± 0.5 °C) after UV−C, $CaCl_2$ (50 mM), and the combined treatment. The treatment methods were the same as those described in Figure 3. The means of three replicates and their SE are presented. There were no significant differences among the treatments at $P < 0.05$.

DISCUSSION

Plant secondary metabolites including Res are unique resources for pharmaceuticals, food additives, and fine chemicals. Elicitors are chemicals or biofactors from various sources that can induce physiological changes of the target living organism. Many studies have shown that some abiotic factors could induce the synthesis of Res.[9,19] In the present study, UV−C largely induced the biosynthesis of Res in leaves and berry skins, and Res content in UV−C leaves and berry skins was about 20-fold higher than that in the control (Figure 2 and Table 2). The results of the expression of stilbene synthesis genes, including *PAL*, *C4H*, *4CL*, *STS*, and *O-3-GT*, significantly increased in leaves after UV−C induction (Figure 4). The peak of expression of the related genes appeared earlier than that of the increases in Res content. Wang et al. showed that UV−C treatment promoted the biosynthesis of Res in leaves and, moreover, significantly promoted the expression of *STS* and protein STS.[9] Takayanagi et al. reported that *PAL*, *C4H*, *4CL*, and *STS* genes were involved in UV−C induced Res biosynthesis in grape berries.[34] The other researchers also reported that some elicitors induced Res biosynthesis in grapevine.[35] Therefore, it should be believed that the UV−C induced accumulation of Res in leaves and in berry skins was achieved through promoting the expression of the related key genes.

Calcium (Ca^{2+}) plays a pivotal role in the physiology and biochemistry of organisms and their cells. Elicitor induced Ca^{2+} flux is important for elicitor induced accumulation of plant secondary metabolites.[36] This dramatic elicitor induced $[Ca^{2+}]$cyt spiking activates many intracellular processes directly or through Ca^{2+} sensors. Zhao et al. showed that the fungal elicitor induced oxidative burst and indole alkaloid accumulation could partially be inhibited by pretreatments of the cell cultures with calcium channel blockers (verapamil and $LaCl_3$) and the Ca^{2+} chelator EGTA or by deleting $CaCl_2$ from the medium, but the inhibition could be overcome by the

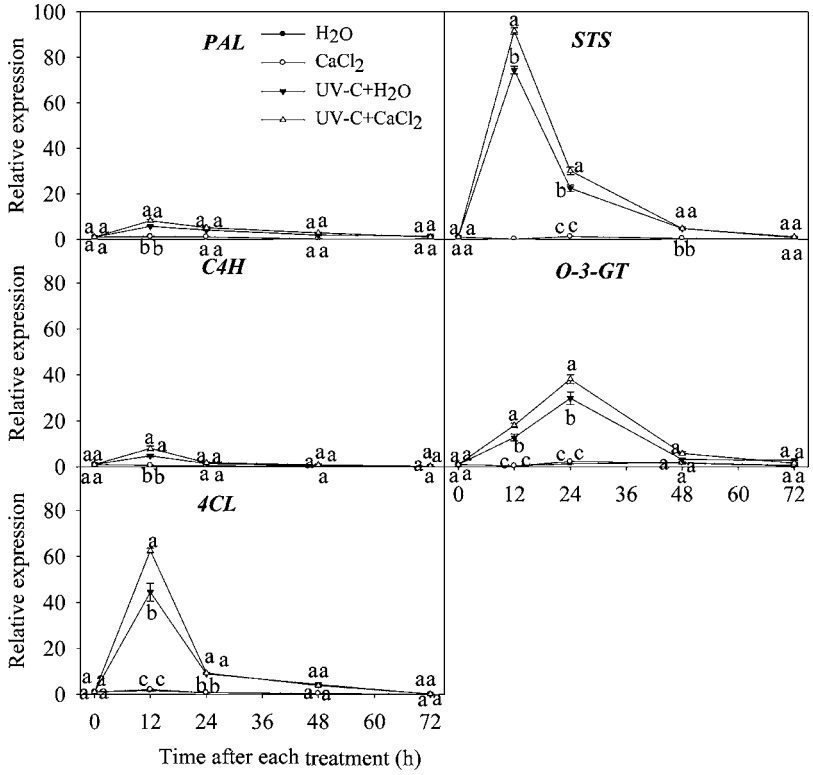

Figure 5. Effect of UV−C, $CaCl_2$ (50 mM), and the combined treatments on expression of mRNA in leaves of 'Beihong'. The treatment methods were the same as those described in Figure 2. The means of three replicates and their SE are presented. Different letters indicate significant differences among the treatments at $P < 0.05$.

readdition of $CaCl_2$.[37] These results suggest that the fungal elicitor triggered indole alkaloid biosynthesis involves Ca^{2+} influx and Ca^{2+} dependent signal transduction. Vandelle et al. suggested that BcPG1 induced stilbene biosynthesis was related to the flux of Ca^{2+} from apoplast to cytoplasm.[38] Shikonin accumulation is related to calcium homeostasis in *Onosma paniculata* cell cultures.[39] The results in the present study showed that exogenous treatments with $CaCl_2$ increased Res content in grape leaves (Figure 1) and in berry skins (Table 2), although the $CaCl_2$ effect on berry skins was much lower than that on leaves. Moreover, the combination treatment of UV−C with $CaCl_2$ resulted in much higher Res content than that in UV−C alone in leaves and berry skins (Figure 1 and Table 2). The expressions of *PAL*, *C4H*, *4CL*, *STS*, and *O-3-GT* in response to UV−C were lower than those in response to UV−C + $CaCl_2$ treatment (Figure 4). It is suggested that Ca^{2+} may take part in the signal transduction pathway of the UV−C induced biosynthesis, which is worth further studying.

Grape is a delicious, nutritious fruit, but it is difficult to store at room temperature. Generally, grape is stored under low temperature and potentially for a long period. Gray mold is the main reason for postharvest decay of table grapes during cold storage and shelf life. The use of ozone and $CaCl_2$ is a promising example of treatments that are beginning to be adopted on a commercial scale.[40] $CaCl_2$ was widely utilized to maintain berry quality, preventing softening, reducing the rate of rot, and prolonging shelf life for harvested fruits and vegetables, including peach, grape, strawberry, pear, and sweet pepper.[26−29,40] Pre- and postharvest application of calcium may help to reduce senescence during commercial and retail storage of fruit, with no detrimental effect on consumer acceptance.[30] Bais et al. also showed that UV−C induced Res biosynthesis in grape berry skin.[15] In the present study, total resveratrol in 'Hongbaladuo' berry skin held at room temperature (24 ± 1 °C) was increased about 7 times by 24 h after UV−C treatment, 20% after $CaCl_2$ treatment, and 11 times after the combination treatment (Table 2). The combination treatment was synergistic in promoting the synthesis of Res compared with that in either treatment alone. Li et al. showed that the *trans*-resveratrol content of UV−C treated grape skin increased during storage.[41] In the present study, the treatment combining UV−C with $CaCl_2$ was the most efficient method in promoting the synthesis of Res, followed by UV−C treatment alone, while the effect of $CaCl_2$ treatment alone was the least. The role of $CaCl_2$ was probably as an important signal molecule to increase UV−C induced resveratrol biosynthesis. Brosche has reported that Ca^{2+} is a part of UV−B induced signal transduction in plants event.[42] During cold storage, the contents of resveratrol in berry skins after UV−C and the combination treatments increased until 13 days, then declined slightly but still remained at high levels at the end of storage. After the 13th day of storage, the resveratrol contents of the UV−C and combination treatments reached the maximum 203.5 and 247.7 μg/g FW, respectively; they then declined slightly to 176.4 and 187.1 μg/g FW by the end of storage (27 days). *trans*-res was the main form of resveratrol in berry skins after the storage treatments, and its response to treatments was similar to that of total resveratrol. *trans*-res accounted for about 90% of the total resveratrol, while *trans*-pd accounted for about 8−10%. UV−C and the combination treatments significantly increased the *trans*-pd contents in skins throughout the storage compared with those in the control. However, *cis*-pd only accounted for a small percentage, about 1% of the total, and all of the treatments had little influence on its accumulation. Storage temperature may influence resveratrol metabolism (decomposition and transformation), but the mechanism(s) need further study. These mechanisms are important for modulating resveratrol content in table grape during storage at low temperature.

The present study showed that UV−C treatment was an efficient approach for inducing the synthesis of resveratrol in grape leaves and berry skins, while $CaCl_2$ could also promote its induction. These results may have application in the production of more resveratrol, which could be good for human health. The results provided good evidence for utilizing grape leaves and developing "functional table grapes".

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Notes

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