Stilbene Compounds and Stilbene Synthase Expression during Ripening, Wilting, and UV Treatment in Grape cv. Corvina

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The content of selected phenolic compounds including resveratrol (*trans*-3,5,4′-trihydroxystilbene) and its *cis*-isomer and glucosides (piceides) were monitored in grape (*Vitis vinifera* L. cv. Corvina) berry skin during ripening in the vineyard and in response to the post-harvest drying process (wilting). Four wilting conditions were compared (traditional, mixed, low-temperature, and high-temperature) to verify the eliciting effect of drying on resveratrol production. During fruit ripening the *cis*-piceid was the major stilbene found in berry skins, and a weak accumulation of stilbene synthase (STS) mRNA was observed, whereas UV-light irradiation greatly stimulated STS transcript of unripe berries. A time-course experiment showed the highest STS mRNA accumulation and resveratrol content (34 μg/g fresh weight at 58 days) occurring in berry skins in a mixed wilting process.

Keywords: *Resveratrol; stilbenes; grape berry; grape skin; ripening; stilbene synthase (STS); UV; wilting*

INTRODUCTION

Plant phenolics have important effects on fruit physiology, as well as on food quality and human nutrition. Their presence in grapes and red wine may contribute to health benefits because of their antioxidant and anticarcinogenic activities (*1, 2*). Resveratrol (*trans*-3,5,4′-trihydroxystilbene), its cis isomeric configurations and their glucosides (piceides) and oligomers (viniferins) are stilbene compounds classified as phytoalexins because of their role in plant defense mechanisms against fungal pathogens (*3−6*).

The synthesis of resveratrol in grape berries and leaves is stimulated by stresses such as fungal infection (mainly *Botrytis cinerea*), injury, and UV light exposure (*7−15*). Moreover, grape cultivar and soil type, as well as enological practices, also affect the final resveratrol content in wine (*16−24*). A high resveratrol concentration in wine has been associated with moderate fungal infection, whereas extensive fungal development may destroy the induced phytoalexin (*25*). In the latter condition the oxidative degradation of resveratrol by both laccase-like stilbene-oxidase of *Botrytis cinerea* (*26*) and grapevine peroxidases (*27*) have been detected.

The free *cis*-resveratrol is rarely monitored in *Vitis vinifera* grape berries. Two hypotheses have been

postulated to explain its origin: (i) the hydrolysis of its *cis*-glucoside (*23*), and (ii) the isomerization of the trans form under UV irradiation (*28*).

The resveratrol synthesis in grape is catalyzed by the stilbene synthase (STS) enzyme which utilizes *p*-coumaryl-CoA and malonyl-CoA as substrates (*29*). The same substrates are also utilized by chalcone synthase (CHS) for the production of chalcone, i.e. the precursor of flavonoids (*30*). The UV irradiation induces resveratrol biosynthesis in grape berry, the final content being related to the developmental stage of the plant (*10*). In fact, the resveratrol content decreases in ripe grape berry exposed to UV irradiation, probably because of the competition between CHS and STS for the same substrate, and the consequent accumulation of anthocyanin in fruits (*31*).

An increased level of STS mRNA induction has been monitored after the addition of fungal cell wall to cell cultures of *Vitis* cv. Optima (*32*). Moreover, elements that are ethylene- and ozone-responsive in the grapevine STS promoter region have been identified by means of reporter genes (*33, 34*). In *Vitis* cv. Optima, the STS enzyme is believed to be encoded by a multigene family of at least seven differentially expressed genes characterized by high nucleotide sequence homology (*32, 35*).

In a previous work, the content of resveratrol isomers in two Valpolicella wines obtained from dried grapes was reported (*36, 37*). Recioto and Amarone are typical Italian wines produced with cv. Corvina grapes from the Valpolicella viticultural area (North Italy). This area is located at low altitude and is not exposed to natural UV irradiation able to induce resveratrol isomerization (*38*). Before winemaking, the picked cluster grapes are layered in plateaus and partially dried in ventilated

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rooms for about 100 days (traditional wilting condition). To reduce the drying time and to minimize the risks of *Botrytis cinerea* infections some alternative wilting procedures, in which grapes are dried by heating, are currently being tested.

This research aims to examine the relationship between the resveratrol content of cv. Corvina grape berry skin and the specific STS enzyme activity during grape ripening and selected post-harvest drying conditions (wilting). The drying process is supposed to be an abiotic stress able to induce stilbene synthase gene expression, and thus, resveratrol production. The induction of STS enzyme in grape skin at different ripening phases, wilting times, and UV treatments has been studied at the transcriptional level by northern blot analysis.

MATERIALS AND METHODS

Sample Collection and Preparation. Healthy grape clusters from cv. Corvina (*Vitis vinifera* L.) were randomly sampled at 2-week intervals from véraison (i.e., the brief period corresponding to the beginning of anthocyanin accumulation in skins of red grape) to ripening. Additional ripe grape clusters were collected and allowed to undergo the post-harvest wilting process. During the grape ripening and wilting processes a sample of 50 fresh random berries was weighed and analyzed for the soluble solids content (°Brix). Hand-peeled berry skins were immediately frozen in liquid nitrogen, stored at −80 °C, and analyzed for resveratrol content, total polyphenols, flavonoids, anthocyanins, catechins, and proanthocyanidins as reported in the following paragraphs.

Wilting Conditions. The ripe grape clusters were dried using one of the following wilting conditions: (a) traditional - the grapes were layered on racks at ambient temperature in a naturally ventilated room for 100 days; (b) mixed - the grapes were heated at 45 °C for 36 h, then placed into the traditional ventilated room for 94 days; (c) low-temperature - the grapes were heated at 28 °C for 15 days; or (d) high-temperature - the grapes were heated at 45 °C for 110 h.

UV Irradiation. The berries from grapes sampled at véraison and ripening were cut from clusters and irradiated with a UV light at 312 nm for 15 min. Control (nonirradiated) and treated berries were incubated in the dark at room temperature with their petioles in water for 24 h. Hand-peeled skins were frozen in liquid nitrogen and stored at −80 °C for HPLC and northern blot analysis.

Analytical Methods. Resveratrol was extracted from berry skins as described by Di Stefano and Cravero (*39*) and analyzed using a Gilson HPLC system (Milano, Italy) equipped with three pumps (mod. 306), a dynamic mixer (mod. 811C), a diode array UV−visible detector (mod. 106), and an injection valve with a 20-μL loop (Rheodyne, Cotati, CA). Separation was carried out at 30 °C on a Spherisorb ODS1 RP-C$_{18}$ column (250 × 4.6 mm, 5 μm particle size) with a precolumn of the same material (Merck, Darmstad, Germany). A ternary gradient elution with acetic acid (A), methanol (B), and water (C) was used as follow: conditioning with 5% A, 10% B, and 85% C (flow rate of 0.4 mL/min); at time 5 min, 5% A, 15% B, and 80% C (flow rate of 0.5 mL/min); and at 30 min, 5% A, 45% B, 50% C (flow 0.5 mL/min) until 45 min. Chromatograms were recorded at 280 and 306 nm. Resveratrol (t_R 36.8 min), *trans*-piceid (t_R 29.2 min), and *cis*-piced (t_R 37.5 min) were identified by their retention times and UV−visible spectra of commercial standard (Sigma, St. Louis, MO), and by comparison with literature data (*25, 28, 36, 40−42*).

Samples of grape berry were also analyzed for total polyphenols (*43*), flavonoids, anthocyanins (*39*), catechins (*44*), and proanthocyanidins (*45*) using spectrophotometric methods.

Isolation of Total RNA. Total RNA was isolated from berry skin according to the procedure described by Loulakakis et al. (*46*) with minor modifications as previously reported (*47*). The RNA concentration and quality were determined by spectrophotometric assay at 260 nm and using the absorbance
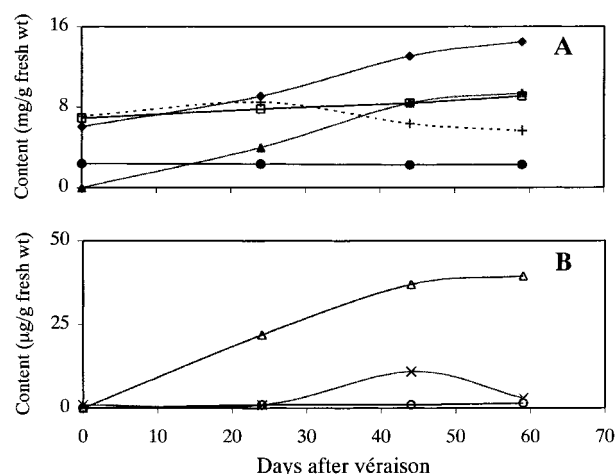

Figure 1. Changes of grape skins (cv. Corvina) during fruit ripening in [A] total polyphenols (□), flavonoids (◆), anthocyanins (▲), catechins (●), and proanthocyanidins (+), and [B] *trans*-resveratrol (○), *trans*-piceid (×), and *cis*-piceid (△). Samples were collected after véraison (time zero).

Table 1. Soluble Solids Content (°Brix) and Berry Weight (g) of cv. Corvina Grapes during Maturation

days from véraison (time zero)	°Brix	berry weight (g)
0	7.3 ± 0.6	1.57 ± 0.08
24	14.1 ± 0.8	1.88 ± 0.11
44	20.4 ± 0.4	1.97 ± 0.20
59	22.1 ± 0.7	2.08 ± 0.15

ratio A$_{260}$/A$_{280}$, respectively (*48*). Moreover, polysaccharide and polyphenol contaminants were checked considering the absorbance ratio A$_{260}$/A$_{230}$ (*49, 50*). The integrity of isolated nucleic acid and its contamination with DNA was examined by agarose gel electrophoresis. In each step, to avoid RNAse contamination, the glassware and solutions were treated following the instructions of Sambrook et al. (*48*).

Northern Blot Analysis. Aliquots of total RNA (12 μg) were denatured and separated on 1.2% (w/v) agarose/formaldehyde gel according to a standard method (*48*). RNAs were blotted onto nylon membrane (Gene Screen Plus, Du Pont, Boston, MA) by capillary transfer using 20× SSC and then fixed by UV cross-linking. The pT7T3 vector containing the pSV368 cDNA clone was double-digested with EcoRI and HindIII restriction enzymes to obtain a fragment of ca. 1370 base pairs. This fragment was ^{32}P labeled by random priming and used as a probe. Hybridization experiment was carried out according to Tonutti et al. (*47*). Two washes were performed at 42 °C using 0.1% SDS added with 1× SSC or 0.1× SSC. Filters were autoradiographed on Kodak X-ray films at −80 °C.

Statistical Analysis. The content of each compound was plotted over the sampling period and the experimental data were fitted by using Statistica 5.1 software (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Changes during Grape Ripening. Grape berries showed good sanitary conditions from véraison to ripening. The berry weight and total soluble content of juice increased throughout ripening up to 2.08 g and 22.1 °Brix, respectively (Table 1). Figure 1 shows the changes of phenolic compounds in grape skin during the development phases. As expected, anthocyanins followed a rising trend, reaching their maximum at ripening, and a similar course was observed for total polyphenols (Figure 1A). Resveratrol was mainly in the glycosilated form (Figure 1B). Its isomer *cis*-piceid was the major

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Table 2. Soluble Solids Content (°Brix) and Berry Weight (g) of cv. Corvina Grapes during Wilting[a]

	wilting conditions[b]			
days	traditional	mixed[c]	low-temp	high-temp
0	22.1[d] [2.08][e]	22.5 [1.63]	22.1 [2.08]	22.1 [2.08]
1.5				22.5 [1.63]
3			22.9 [1.96]	
4.6				25.1 [1.40]
15			27.4 [1.54]	
30		22.4 [1.65]		
40	25.9 [1.73]			
58		26.3 [1.28]		
74	27.1 [1.61]			
94		26.2 [1.17]		
100	28.6 [1.69]			

[a] Results are reported as °Brix [berry weight (g)]. [b] Wilting conditions: traditional (at room temperature); mixed (at 45 °C × 36 h; then 94 days at r.t.); low-temp (at 28 °C × 15 days); high-temp (at 45 °C × 110 h, i.e., 4.6 days). [c] Days represent the duration of wilting at room temperature (after the drying at 45 °C × 36 h). [d] The mean values are tabled. The pooled SD is as follows: traditional (0.85); mixed (1.14); low-temperature (0.81); high-temperature (0.72). [e] The mean values are tabled. The pooled SD is as follows: traditional (0.14); mixed (0.22); low-temperature (0.13); high-temperature (0.12).

stilbene found in berry skins, and it showed a steady increase during fruit ripening (39.5 μg/g fw at 60 days after véraison). Conversely, resveratrol was not detectable at véraison and reached only 1.5 μg/g fw at ripening. These findings confirm that resveratrol is mostly bound as *cis*- and *trans*-glucosides in grape berry skins (*19, 51*) and suggest that a high resveratrol content may be related to gray mold infection. The total resveratrol content found in this study increased during grape ripening and its level was in agreement with previous findings (*37*).

Changes during Grape Wilting. The drying process of grapes was followed by monitoring berry weight and soluble solid content (Table 2). Traditional wilting increased the °Brix value of grape berries up to 28.6, whereas berry weight was reduced by 0.47 g. However, the maximum weight loss (0.68 g) was obtained with the high-temperature condition. Total polyphenols, flavonoids, anthocyanins, catechins, and proanthocyanidins contents of grape berry skin slightly decreased under mixed wilting (Figure 2A), whereas their concentrations remained almost stable using the other wilting conditions tested (data not shown).

During wilting the content of stilbenes always increased with respect to time zero, except for the high-temperature condition. In particular, by using mixed wilting the resveratrol content peaked at 35 μg/g fw at day 58 (Figure 2B) and a similar trend was observed under traditional wilting for resveratrol (28 μg/g fw at day 74) and its *trans*-piceid (15 μg/g fw at day 74). After an initial increase there was a slight decrease of resveratrol content toward the end of the drying processes that might indicate stabilization or fall. The combined effect of time and temperature seems to play an important role as a stress factor in resveratrol accumulation during post-harvest of grape berry. Cantos et al. (*15*) reported no significant difference in resveratrol content between control and UV-treated grapes during storage at 0 °C for 10 days. However, when grapes were transferred to 15 °C for 5 days a significant difference was observed between the control and the irradiated samples.

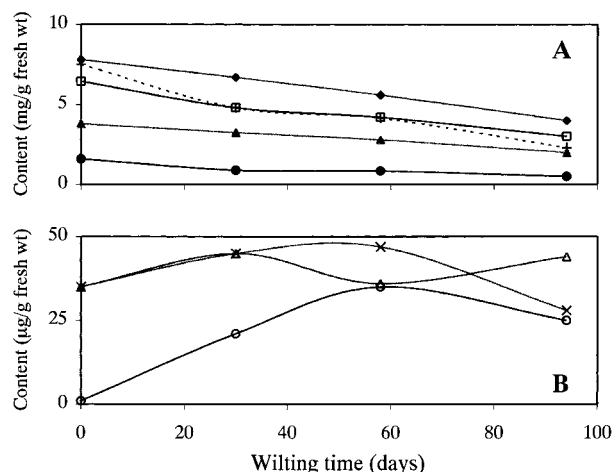**STS mRNA Induction by UV Treatment during Ripening.** The hybridization of total RNA with the

Figure 2. Changes of grape skins (cv. Corvina) during fruit wilting under mixed wilting condition (at 28 °C for 15 days) in [A] total polyphenols (□), flavonoids (◆), anthocyanins (▲), catechins (●), proanthocyanidins (+), and [B] *trans*-resveratrol (○), *trans*-piceid (×), and *cis*-piceid (△) .

pSV368 cDNA clone identified a STS transcript of 1.7 Kb. Only a minor difference in STS induction was monitored during the 59 days of ripening (Figure 3A), thus confirming the results reported for resveratrol content in the same period. The effect of an UV elicitation treatment during ripening showed interesting results (Figure 3B). In fact, the UV exposure (15 min) of berry skin at véraison induced a high STS signal coupled with a noticeable increase in resveratrol content (39.8 μg/g fw). Conversely, it appeared that the UV exposure did not have the same effect on grape skin at harvest (i.e., after 59 days of ripening) as demonstrated by the resveratrol level (4.8 μg/g fw) and STS mRNA accumulation.

Effect of Wilting on STS mRNA Induction. High STS mRNA induction and resveratrol content in berry skin were reached after 74 days during traditional wilting (Figure 4A). However, the highest levels were monitored at 58 days during a mixed wilting (Figure 4B); the mixed wilted sample showed a low mRNA inhibition effect at 94 days, whereas both wilting processes showed a similar decrease in resveratrol content.

Because resveratrol is a phytoalexin that is also synthesized in response to biotic stress, it could be argued that its high content in berry skin might be a response to *Botrytis cinerea* attach occurring during wilting. The good sanitary condition of the grapes monitored throughout all of the experiments brought us to exclude this hypothesis, unless an undetectable larval form was present.

In the attempt to get more insight into the effect of temperature and duration of drying treatment on the molecular mechanism of resveratrol synthesis, the STS mRNA levels of berry skin wilted at different conditions were also studied. During low-temperature wilting (15 days at 28 °C) a weak STS signal was detected only upon 15 days of treatment (Figure 4C). Figure 2D shows that the opposite trend was observed when a high-temperature wilting (110 h at 45 °C) was performed. In this case the transcriptional signal of pSV368, as well as the resveratrol content, progressively decreased, reaching their relative minimums after 110 h.

Taken together, these results showed that resveratrol content in grape skin of cv. Corvina was coupled with
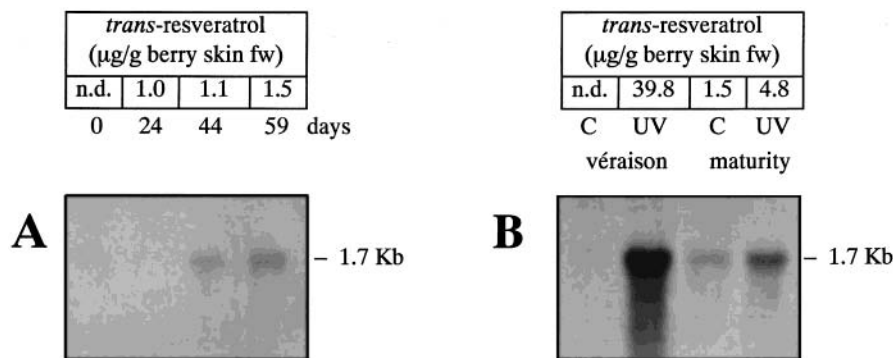
trans-resveratrol (µg/g berry skin fw)			
n.d.	1.0	1.1	1.5
0	24	44	59 days

trans-resveratrol (µg/g berry skin fw)			
n.d.	39.8	1.5	4.8
C	UV	C	UV
	véraison		maturity

Figure 3. Northern blot hybridization of total RNA isolated from grape skin of cv. Corvina at different ripening phases and after UV exposure probed with pSV368 cDNA clone. STS mRNA induction and *trans*-resveratrol content (A) during ripening, and (B) at véraison and full ripening in UV-light exposed and unexposed skin grape at véraison and harvest. *trans*-Resveratrol content is expressed as µg/g fresh weight.
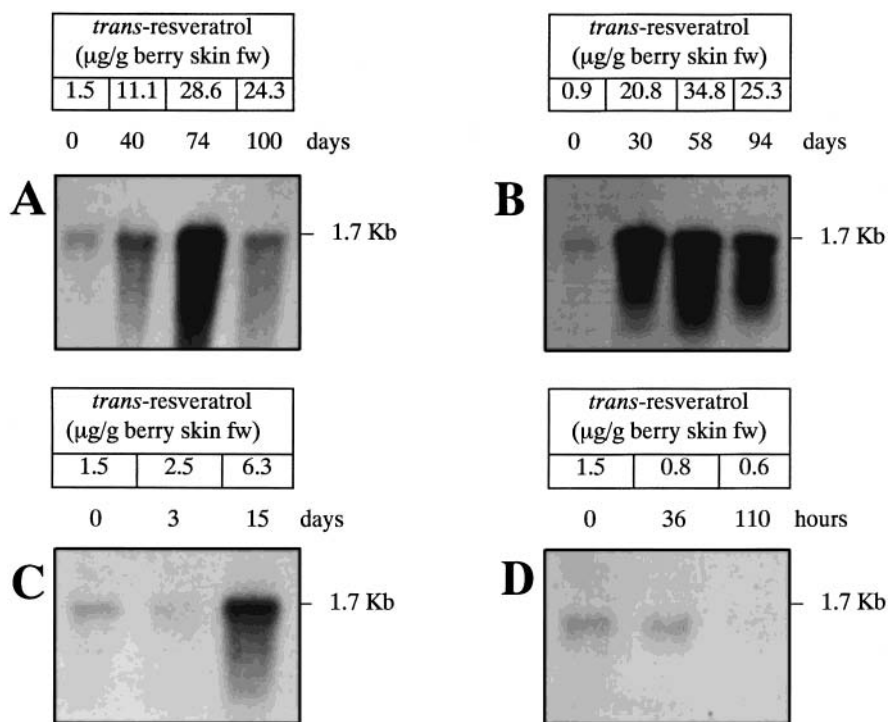
trans-resveratrol (µg/g berry skin fw)			
1.5	11.1	28.6	24.3
0	40	74	100 days

trans-resveratrol (µg/g berry skin fw)			
0.9	20.8	34.8	25.3
0	30	58	94 days

trans-resveratrol (µg/g berry skin fw)		
1.5	2.5	6.3
0	3	15 days

trans-resveratrol (µg/g berry skin fw)		
1.5	0.8	0.6
0	36	110 hours

Figure 4. Time-course experiment of STS mRNA induction and *trans*-resveratrol content (µg/g fresh weight) in grape skin under four different wilting conditions; (A) traditional - grape layered in a naturally ventilated room for 100 days; (B) mixed - induction in skin grape heated for 36 h at 45 °C then placed into the traditional ventilated room for 94 days; (C) low-temperature - heated for 15 days at 28 °C; (D) high-temperature - heated at 45 °C for 110 h.

an increased STS mRNA induction. In particular, the UV exposure and certain wilting processes may positively influence the STS transcription level and, as a consequence, the resveratrol content. An important finding of this study was that following UV irradiation the resveratrol concentration increased by a factor of ca. 40 in grape skin at véraison (Figure 3B) but not at ripening. Moreover, mixed wilting greatly anticipated high STS mRNA induction and resveratrol synthesis when compared to the traditional wilting (Figures 4A and 4B). On the other hand, short-term associated with high-temperature wilting did not seem to produce the same effect (Figure 4D).

It has been reported that in *Vitis* cv. Optima the resveratrol synthase is most likely organized by a complex of at least seven genes and that mRNAs of six genes are differentially accumulated during 12 h elicitor-fungal attack (*35*), thus suggesting a different involvement of the isolated genes in resveratrol synthesis and induction of STS activity. The cDNA se-

quence contained in the pSV368 clone is able to hybridize a transcript which is differentially regulated according to the developmental phase of the grape berry and has been demonstrated to actively participate in de novo synthesis of resveratrol during UV exposure and the wilting process. The noticeable induction of STS mRNA and the consistent increase of resveratrol at véraison was not found at harvest (Figure 1B); these findings support the already formulated hypothesis of a more active resveratrol synthesis from véraison until the phase which precedes ripening, at which time the ability to synthesize resveratrol progressively decreases until the complete ripening of the berry (*9, 11, 52*). It has been reported that the decreased ability of grape skin to synthesize resveratrol at ripening in response to UV irradiation may be due to an increased chalcone synthase (CHS) activity (which utilizes the same substrate as stilbene synthase) with accumulation of anthocyanin (*31*). The obtained results suggest that it is not the competition between CHS and STS for the same sub-

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strate which determines resveratrol decrease, but the lowered sensitivity of the ripe berry to UV treatment, thus determining only a weak STS mRNA induction and resveratrol content.

The results presented in this study indicate that UV irradiation and the wilting process play different roles in the STS induction, thus suggesting the presence of mechanisms which differentially modulate STS mRNA induction and resveratrol synthesis. In particular, mixed wilting is a method of choice to increase expression and accumulation of resveratrol in wine. To understand more about the molecular mechanism regulating the resveratrol synthesis, and the role of each isolated gene involved in stilbene synthase activity, it would be interesting to investigate the STS induction level of the sequence specific for the seven genes belonging to the family under the same conditions monitored in this study. Moreover, deletion and overexpression of each STS gene might contribute to clarifying their role in stilbene synthase activity and resveratrol synthesis. More information will be useful to understanding which sequences are involved in activation or inactivation/ degradation of the stilbene synthase in different steps or grape developmental phases.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidant in grapes and wines. *J. Agric. Food Chem.* **1994**, *42*, 64−69.

(2) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Resveratrol: a molecule whose time has come? And gone? *Clin. Biochem.* **1997**, *30*, 91−113.

(3) Hart, J. H. Role of phytostilbene in decay and disease resistance. *Annu. Rev. Phytopathol.* **1981**, *19*, 437−458.

(4) Langcake, P. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ϵ-viniferin, α-viniferin and pterostilbene. *Physiol. Plant Pathol.* **1981**, *18*, 213−226.

(5) Hain, R.; Bieseler, B.; Kindl, H.; Schröder, G.; Stöcker, R. Expression of a stilbene synthase gene in *Nicotiana tabacum* results in synthesis of the phytoalexin resveratrol. *Plant Mol. Biol.* **1990**, *15*, 325−335.

(6) Fischer, R.; Hain, R. Plant disease resistance resulting from the expression of foreign phytoalexins. *Curr. Opin. Biotechnol.* **1994**, *5*, 125−130.

(7) Langcake, P.; Pryce, R. J. The production of resveratrol by *Vitis vinifera* and other membres of the *Vitaceae* as a response to infection or injury. *Physiol. Plant Pathol.* **1976**, *9*, 77−86.

(8) Langcake, P.; McCarthy, W. V. The relationship of resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. *Vitis* **1979**, *18*, 244−253.

(9) Creasy, L. L.; Coffee, M. Phytoalexin production potential of grape berries. *J. Am. Soc. Hortic. Sci.* **1988**, *113*, 230−234.

(10) Jeandet, P.; Bessis, R.; Gautheron, B. The production of resveratrol (3, 5, 4′-trihydroxystilbene) by grape berries in different developmental stages. *Am. J. Enol. Vitic.* **1991**, *42*, 41−46.

(11) Liswidowati, M. F.; Hohmann, F.; Burkhardt, S.; Kindl, H Induction of stilbene synthase by *Botrytis cinerea* in cultured grapevine cells. *Planta* **1991**, *183*, 307−314.

(12) Jeandet, P.; Bessis R.; Sbaghi, M.; Meunier, P. Production of the phytoalexin resveratrol by grape as a response to *Botrytis cinerea* attacks under natural conditions. *J. Phytopathol.* **1995**, *143*, 135−139.

(13) Adrian, M.; Jeandet, A. C.; Douillet-Breuil, L.; Bessis, R. Stilbene content of mature *Vitis vinifera* berries in response to UV−C elicitation. *J. Agric. Food Chem.* **2000**, *48*, 6103−6105.

(14) Bais, A. J.; Murphy, P. J.; Dry, I. B. The molecular regulation of stilbene phytoalexin biosynthesis in *Vitis vinifera* during grape berry development. *Aust. J. Plant Physiol.* **2000**, *27*, 425−433.

(15) Cantos, E.; García-Viguera, C.; de Pascual-Teresa, S.; Tomás-Barberán, F. A. Effect of postharvest ultraviolet irradiation on resveratrol and other phenolics of cv. Napoleon table grapes. *J. Agric. Food Chem.* **2000**, *48*, 4606−4612.

(16) Siemann, E. H.; Creasy, L. L. Concentration of the phytoalexyn resveratrol in wine. *Am. J. Enol. Vitic.* **1992**, *43*, 49−52.

(17) Jeandet, P.; Bessis, R.; Maume, B. F.; Sbaghi, M. Analysis of resveratrol in Burgundy wines. *J. Wine Res.* **1993**, *4*, 79−85.

(18) Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, E. P.; Karumanchiri, A.; Soleas G. J.; Waterhouse, A. L. A global survey of *trans*-resveratrol concentrations in commercial wines. *Am. J. Enol. Vitic.* **1995**, *46*, 159−165.

(19) Mattivi, F.; Reniero, F.; Korhammer S. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **1995**, *43*, 1820−1823.

(20) Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Diamandis, E. P.; Soleas, G. J. Resveratrol glucosides are important components of commercial wines. *Am. J. Enol. Vitic.* **1996**, *47*, 415−420.

(21) Pezet, R.; Cuneat, Ph. Resveratrol in wine: extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.* **1996**, *47*, 287−290.

(22) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Levels of *cis*- and *trans*-resveratrol and their glucosides in white and rosé *Vitis vinifera* wines from Spain. *J. Agric. Food Chem.* **1996**, *44*, 2124−2128.

(23) Vrhovsek, U.; Wendelin, S.; Eder, R. Effects of various vinification techniques on the concentrations of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. *Am. J. Enol. Vitic.* **1997**, *48*, 214−219.

(24) Threlfall, R. T.; Morris, J. R.; Mauromoustakos, A. Effect of variety, ultraviolet light exposure, and enological methods on the *trans*-resveratrol level of wine. *Am. J. Enol. Vitic.* **1999**, *50*, 57−64.

(25) Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. Resveratrol content of wines of different ages: relationship with fungal disease pressure in the vineyard. *Am. J. Enol. Vitic.* **1995**, *46*, 1−4.

(26) Breuil, A. C.; Adrian, M.; Pirio, N.; Meunier, P.; Bessis, R.; Jeandet, P. Metabolism of stilbene phytoalexins by *Botrytis cinerea*: 1. Characterization of a resveratrol dehydrodimer. *Tetrahedron Lett.* **1998**, *39*, 537−540.

(27) Morales, M.; Alcántara, J.; Barceló A. R. Oxidation of *trans*-resveratrol by a hypodermal peroxidase isoenzyme from Gamay rouge grape (*Vitis vinifera*) berries. *Am. J. Enol. Vitic.* **1997**, *48*, 33−38.

(28) Roggero, J. P.; Garcia-Parrilla, C. Effects of ultraviolet irradiation on resveratrol and changes in resveratrol and various of its derivatives in the skins of ripening grapes. *Sci. Aliments* **1995**, *15*, 411−422.

(29) Fritzemeier, K. H.; Kindl, H. Coordinate induction by UV light of stilbene synthase phenylalanine ammonialyase and cinnamate 4-hydroxylyase in leaves of *Vitaceae*. *Planta* **1981**, *151*, 48−52.

(30) Melchior, F.; Kindl, H. Grapevine stilbene synthase cDNA only slightly differing from chalcone synthase cDNA is expressed in *Escherichia coli* into a catalytically active enzyme. *FEBS Lett.* **1990**, *268*, 17−20.

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(31) Jeandet, P.; Sbaghi, M.; Bessis, R.; Meunier, P. The potential relationship of stilbene (resveratrol) synthesis to anthocyanin content in grape berry skin. *Vitis* **1995**, *2*, 91−94.

(32) Melchior, F.; Kindl, H. Coordinated and elicitor-dependent expression of stilbene synthase and phenylalanine ammonia-lyase genes in *Vitis* cv. Optima. *Arch. Biochem. Biophys.* **1991**, *288*, 552−557.

(33) Grimming, B.; Schubert, R.; Fischer, R.; Hain, R.; Schreier, P. H.; Langebartels, C.; Ernst, D.; Sandermann, H. Jr. Ozone and ethylene-induced regulation of a grapevine resveratrol synthase promoter in transgenic tobacco. *Acta Physiol. Plant.* **1997**, *19*, 467−474.

(34) Schubert, R.; Fischer, R.; Hain, R.; Schreirer, P. H.; Bahnweg, G.; Ernst, D.; Sandermann, H. Jr. An ozone-responsive region of the grapevine resveratrol synthase promoter differs from the basal pathogen-responsive sequence. *Plant Mol. Biol.* **1997**, *34*, 417−426.

(35) Wiese, W.; Vornam, B.; Krause, E.; Kindl, H. Structural organization and differential expression of three stilbene synthase genes located on a 13 kb grapevine DNA fragment. *Plant Mol. Biol.* **1994**, *26*, 667−677.

(36) Celotti, E.; Ferrarini, R.; Zironi, R.; Conte, L. S. Resveratrol content of some wines obtained from dried Valpolicella grapes: Recioto and Amarone. *J. Chromatogr. A* **1996**, *730*, 47−52.

(37) Celotti, E.; Ferrarini, R.; Conte, L. S.; Giulivo, C.; Zironi, R. Modifiche del contenuto di resveratrolo in uve di vitigni della Valpolicella nel corso della maturazione e dell'appassimento. *Vignevini* **1998**, *5*, 83−92.

(38) Fregoni, M.; Bavaresco, L.; Petegolli, D.; Trevisan, M. Indagine sul contenuto di resveratrolo in alcuni vini della Valle d'Aosta e dei Colli Piacentini. *Vignevini* **1994**, *21*, 33−36.

(39) Di Stefano, R.; Cravero, M. C. Metodi per lo studio dei polifenoli nell'uva. *Riv. Vitic. Enol.* **1991**, *2*, 37−45.

(40) Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Yan, J.; Diamandis, E. P.; Soleas G. J. Assay of resveratrol glucosides and isomers in wine by direct-injection high-performance liquid chromatography. *J. Chromatogr. A* **1995**, *708*, 89−98.

(41) Lamuela-Raventós, R. M.; Romero-Prez, A. I.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Direct HPLC analysis of *cis-* and *trans-*resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **1995**, *43*, 281−283.

(42) Barbanti, D.; Galassi, S.; Versari, A.; Burattini, R. Gas chromatography and high-pressure liquid chromatography determination of resveratrol in Italian red wines. *J. Wine Res.* **1996**, *7*, 5−11.

(43) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−159.

(44) Di Stefano, R.; Cravero, M. C.; Gentilini, N. Metodi per lo studio dei polifenoli nei vini. *Enotecnico* **1989**, *25*, 83−89.

(45) Margheri, G.; Falceri, E. Importanza dell'evoluzione delle sostanze polifenoliche nei vini rossi di qualità durante l'invecchiamento. *Vini Ital.* **1972**, *81*, 501−511.

(46) Loulakakis, K. A.; Roubelakis-Angelakis, K. A.; Kanellis, A. K. Isolation of functional RNA from grapevine tissues poor in nucleic acid content. *Am. J. Enol. Vitic.* **1996**, *47*, 181−185.

(47) Tonutti, P.; Bonghi, C.; Ruperti, B.; Tonielli, G. B.; Ramina, A. Ethylene evolution and 1-aminocyclopropane-1-carboxylate oxidase gene expression during early development and ripening of peach fruit. *J. Am. Soc. Hortic. Sci.* **1997**, *122*, 642−647.

(48) Sambrook, J.; Fritsch, E. F.; Maniatis, F. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

(49) Logemann, J.; Schell, J.; Willmitzer, L. Improved method for the isolation of RNA from plant tissues. *Anal. Biochem.* **1986**, *163*, 16−20.

(50) Manning, K. Isolation of nucleic acids from plants by differential solvent precipitation. *Anal. Biochem.* **1991**, *195*, 45−50.

(51) Waterhouse, A. L.; Lamuela-Raventós, R. Ma. The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochemistry* **1994**, *27*, 571−573.

(52) Bavaresco, L.; Pettegolli, D.; Cantù, E.; Fregoni, M.; Chiusa, G.; Trevisan, M. Elicitation and accumulation of stilbene phytoalexin in grapevine berries infected by *Botrytis cinerea*. *Vitis* **1997**, *36*, 77−83.