Euphytica (2010) 176:371–381
DOI 10.1007/s10681-010-0221-0

Resveratrol production potential of grape flowers and green berries to screen genotypes for gray mold and powdery mildew resistance

Mikio Shiraishi · Hiroyuki Chijiwa · Hiroyuki Fujishima · Koji Muramoto

Received: 26 March 2010 / Accepted: 5 July 2010 / Published online: 14 July 2010

Abstract The ability of grape cultivars to produce resveratrol in response to UV radiation is associated with their resistance to fungal pathogens. In this study, we evaluated the UV-induced resveratrol production potential of grape flowers and green berries of 72 grape genotypes. Their ability to produce resveratrol in response to UV radiation was used to establish a selection criterion for screening genotypes for resistance to gray mold and powdery mildew. We quantified resveratrol in grape flower extracts from pre-bloom and early bloom flowers after exposure to UV radiation. There was a strong negative correlation between UV-induced resveratrol production and susceptibility to *Botrytis* infection. The grape genotype was considered to be susceptible to gray mold when the resveratrol content of whole flower clusters was less than 10 μg g^{-1} FW after the UV treatment. We determined resveratrol production in response to UV radiation in whole grape berries from 0 to 30 days after full bloom. The ability to produce resveratrol in response to UV increased by 8- to 20-times during this period, depending on the genotype. At 30 days after full bloom, *Vitis vinifera* genotypes generally had low levels of resveratrol (<50 μg g^{-1} FW), while interspecific hybrids, especially the North American cultivars, had high levels (>50 μg g^{-1} FW). There were clear varietal differences in resistance to *Erysiphe* (powdery mildew) infection. Susceptibility to *Erysiphe* infection was strongly negatively correlated with UV-induced resveratrol production.

Keywords Breeding · Disease resistance · Grape · Gray mold · Powdery mildew · Resveratrol

M. Shiraishi (✉) · H. Chijiwa · H. Fujishima · K. Muramoto
Fukuoka Agricultural Research Center, 587 Yoshiki, Fukuoka 818-8549, Japan
e-mail: mikioshi@farc.pref.fukuoka.jp

Introduction

Gray mold (*Botrytis cinerea* Pers.) and powdery mildew (*Erysiphe necator* Schwein) are widespread fungal diseases of field- and greenhouse-grown grapevines in temperate areas (Pearson and Goheen 1994; Reisch and Pratt 1996). In viticulture, serious economic losses result from the effects of these fungal infections on the fruit, e.g., poor fruit set, premature drop of bunches, uneven maturation, cracking that leads to fruit rot, and decreased yields. In Japan, fungicides are applied at high levels to grapes from the bloom stage to the green berries stage to control these diseases, especially in greenhouse-grown grapevines. Breeding grape cultivars with innate resistance to these fungal diseases could reduce the costs of chemical control.

In grapevines, disease resistance involves both structural and biochemical defense mechanisms. One

of these mechanisms, the production of the stilbene-type phytoalexin, *trans*-resveratrol (hereafter resveratrol), is particularly effective against fungal diseases, as it inhibits spore germination and growth of the fungal pathogen (Dercks et al. 1995). Resveratrol production of grape leaves in response to fungal attacks or short ultraviolet (UV)-treatments has been extensively studied (Langcake and Pryce 1977; Langcake and McCarthy 1979; Langcake 1981; Pool et al. 1981; Stein and Blaich 1985; Barlass et al. 1987; Bavaresco and Eibach 1987; Dercks and Creasy 1989; Adrian et al. 1997). The results of these studies indicated that the capacity for resveratrol production is positively correlated with disease resistance of grapevines.

Ultraviolet radiation is a useful induction technique, because it induces resveratrol synthesis in a large proportion of exposed cells. Thus, the potential for resveratrol production in response to UV radiation may be a useful marker in assessing resistance of flowers/green berries to diseases, as was the case for predicting disease resistance of leaf tissue (Sbaghi et al. 1995). However, there are few studies on resveratrol production potential of grape flowers and green berries (Creasy and Coffee 1988; Jeandet et al. 1991; Bavaresco et al. 1997; Keller et al. 2003). If there are varietal differences in the potential for resveratrol production in flowers and green berries, this could be used to screen grape genotypes for disease resistance. Screening procedures are a crucial part of any breeding program, and must be simple and rapid. The purpose of this study was (1) to evaluate UV-induced resveratrol production in grape flowers and green berries, and (2) to use these results to establish a selection criterion for screening grapevine genotypes (table and wine cultivars) for resistance to gray mold and powdery mildew.

Materials and methods

Plant material

We used 72 grape genotypes, as follows (Table 1): 48 *Vitis labruscana* × *Vitis vinifera* interspecific hybrids (Aki Queen, Akitsu No. 27, Alden 4X, Black Beat, Buffalo 4X, Campbell Early, Delaware, Festivee, Fujiminori, Fukuoka No. 12, Gorby, Honey Venus, Houman, Kyoho, Kai Noir, Muscat Bailey A, Neo Alicante, Niagara Rosada, Ohtama Portland, Olympia, Oriental Star, Pierce, Pione, Portland, Red Port, Red Port 4X, Rubired, Schuyler 4X, Shine Muscat, Steuben, Steuben 4X, Suiho, Sunny Rouge, TSA-4, Wayne 4X, Yoho, 3891, 5512, 95-13-2, 02-2-38, 02-3-24, 02-3-32, 02-7-2, 02-9-4, 02-9-13, 02-10-2, 02-12-44, and 03-6-1); 22 *V. vinifera* genotypes (Alicante Bouschet, Bijou Noir, Cabernet Sauvignon, French Colombard, Fukuoka No. 13, Fukuoka No. 14, Hakata White, Harmo Noir; Merlot, Muscat of Alexandria, Muscat Hamburg, Neo Muscat, Palestine, Red Ohanez, Rosario Bianco, Ruby Okuyama, Ruby Seedless, Sunny Dolce, Syrah, Yatomi Rosa, 6706, and 97-1-14); and two rootstocks (Kober 5BB and Riparia Gloire de Montpellier). All genotypes were grown with a vine-by-row spacing of 0.8 × 2.0 m in a North–South orientation in a greenhouse at the Fukuoka Agricultural Research Center, Fukuoka, Japan. We used one self-rooted vine per genotype. The vines were cane-pruned to 15–20 nodes and trained onto a horizontal trellis system. Fungicides were applied to prevent latent infection with gray mold and powdery mildew before flower or berry sampling. The spraying regime was as follows: at the five- to six-leaves unfolded stage, we applied famoxadone; at the beginning of flowering, iprodione; at late flowering, diethofencarb; at fruit set, azoxystrobin; and when berries were pea-sized, triflumizole. These fungicide applications were carried out for all experiments except for experiment 5, in which we evaluated natural infection of grapevines by *E. necator*.

Sample preparation of flower clusters and green berries

Detached clusters were washed in running water for 1 h. After surface sterilization (70% ethanol, 30 s) and rinsing in sterile water, three clusters were placed on a Petri dish (ϕ 150 × 15 mm) containing moist filter paper. In the following experiments, we used three to five Petri dishes (replicates) of each genotype for UV irradiation and *Botrytis* inoculation.

Gray mold experiments

Experiment 1

In 2007, we sampled eight American hybrids and one *vinifera* genotype to determine resveratrol production

Table 1 Origin and use of 72 grape genotypes used in this study

Origin (country)	Cultivar or selection (use)
Egypt	Muscat of Alexandria* (TW)
Syria	Palestine* (T)
United Kingdom	Muscat Hamburg* (T)
Austria	Kober 5BB (R)
France	Alicante Bouschet* (W); Cabernet Sauvignon* (W); French Colombard* (W); Merlot* (W); Riparia Gloire de Montpellier (R); Syrah* (W)
Russia	Red Ohanez* (T)
United States	Campbell Early (T); Delaware (T); Festivee (T); Pierce (T); Portland (T); Rubired (W); Ruby Seedless* (T); Steuben (T)
Brazil	Niagara Rosada (T)
Japan	Aki Queen (T); Akitsu No. 27 (T); Alden 4X (T); Bijou Noir* (W); Black Beat (T); Buffalo 4X (T); Fujiminori (T); Fukuoka No. 12 (T); Fukuoka No. 13* (T); Fukuoka No. 14* (T); Gorby (T); Hakata White (T); Harmo Noir* (W); Honey Venus (T); Houman (T); Kai Noir (W); Kyoho (T); Muscat Bailey A (TW); Neo Alicante (W); Neo Muscat* (T); Ohtama Portland (T); Olympia (T); Oriental Star (T); Pione (T); Red Port (T); Red Port 4X (T); Rosario Bianco* (T); Ruby Okuyama* (T); Shine Muscat (T); Schuyler 4X (T); Steuben 4X (T); Suiho (T); Sunny Dolce* (T); Sunny Rouge (T); TSA-4 (T); Wayne 4X (T); Yatomi Rosa* (T); Yoho (T); 02-2-38 (T); 02-3-24 (T); 02-3-32 (T); 02-7-2 (T); 02-9-4 (T); 02-10-2 (T); 02-12-44 (T); 02-9-13 (T); 03-6-1 (T); 3891 (T); 5512 (T); 6706* (T); 95-13-2 (T); 97-1-14* (T)

Genotypes are derived from *Vitis vinifera** or interspecific hybrid (*V. labruscana* × *V. vinifera*)

T table grapes, *W* wine grapes, *R* rootstock (Kober 5BB: *V. berlandieri* × *V. riparia*, Riparia Gloire de Montpellier: *V. riparia*)

and *Botrytis* resistance at the pre-bloom stage. Unopened flower clusters were sampled at stage 17 (fully developed; flowers separating) as defined by Eichhorn and Lorenz (1977). The detached clusters (three replicates for each cultivar) were irradiated for 15 min at a distance of 10 cm with a 254 nm wavelength UV lamp (SUV-16, 16 W, 2020 μW cm^{-2} at 5 cm, AS ONE, Osaka, Japan) in a dark room at 25°C. After UV irradiation, the clusters were enclosed in a polyethylene bag (0.03 × 260 × 380 mm), kept in the dark at 25°C for 48 h (Pool et al. 1981), and then immediately frozen at −20°C until resveratrol analysis. For the *Botrytis* inoculation, clusters from five replicates were dipped in *Botrytis* conidial suspension (10^5 spores ml^{-1}). After inoculation, the clusters were placed in a Petri dish and enclosed in a polyethylene bag as above, and kept in the dark at 20°C for 5 days.

Experiment 2

In 2007, we sampled four interspecific hybrids and one *vinifera* genotype to determine resveratrol production and *Botrytis* resistance at the early bloom stage. Opened flower clusters were sampled at stage 21 (25% of caps fallen) as defined by Eichhorn and Lorenz (1977). The detached clusters (five replicates of each genotype, including controls) were UV irradiated and incubated as described above. After incubation, clusters were immediately frozen at −20°C until resveratrol analysis. For the *Botrytis* inoculation, clusters (five replicates per cultivar) were inoculated and incubated as described in experiment 1.

Experiment 3

In 2008, we determined resveratrol production at early bloom in 69 genotypes (46 interspecific hybrids, 21 *vinifera* genotypes, and two rootstocks). In each genotype, five replicates of detached clusters were UV irradiated and incubated as described in experiment 1. After incubation, the clusters were immediately frozen at −20°C until resveratrol analysis.

B. cinerea cultures and preparation of conidial suspension

B. cinerea (strain BCT01, MAFF 665011) was supplied by Dr. R. Nakaune (Grape and Persimmon Research Station, NIFTS, Higashi-Hiroshima, Japan). Fungal colonies were cultured on potato dextrose agar

at 20°C in the dark for 72 h. Sporulation was induced by BLB irradiation (365 nm, 4 W, 513 μW cm^{-2} at 5 cm) at a distance of 10 cm for 48 h, then incubation for 48 h. A conidial suspension was prepared from these 7-day-old cultures by flooding the Petri dishes with sterile potato dextrose solution (0.2% potato starch, 1.0% dextrose, 0.01% Tween 20) and scraping the agar surface to dislodge conidia. The conidial suspension was obtained by filtering the mixture through two layers of cheese cloth.

Visual assessment of symptoms

Disease symptoms on the clusters were classified according to the following scale: 0 = no symptoms; 1 = one to two pedicels infected; 3 = three to four pedicels infected; 5 = more than five pedicels infected. The infection index for *Botrytis* (*IIB*, %) was calculated as follows:

$$IIB = \left(\left[\sum_{i=0}^{5} n_i \times i \right] \Big/ \left[\sum_{i=0}^{5} n_i \times 5 \right] \right) \times 100$$

where i = the infection scale rating (0–5) and n = the number of clusters in each infection class.

Powdery mildew experiments

Experiment 4

In 2007, we selected three interspecific hybrid genotypes to monitor changes in UV-induced resveratrol production after full bloom. The clusters were sampled at 0, 15, and 30 days after full bloom, and were UV irradiated as described in experiment 1.

Experiment 5

In 2008, we examined resveratrol production in grape berries at 30 days after full bloom. For this analysis, we selected 68 genotypes (46 interspecific hybrids, 21 *vinifera* genotypes, and 1 rootstock). We selected 6–10 berries, depending on berry size, for UV irradiation using the procedures described in experiment 1. The remaining fresh berry samples were crushed in a hand press, and the percentage of soluble solids in the juice was measured with a refractometer (N1, ATAGO, Japan).

Visual assessment of symptoms

A total of 15 genotypes (Alden 4X, Buffalo 4X, Campbell Early, Delaware, Festivee, French Colombard, Muscat of Alexandria, Muscat Hamburg, Portland, Red Ohanez, Rubired, Ruby Okuyama, Ruby Seedless, Schuyler 4X, and Steuben 4X) were naturally infected by *E. necator* in the isolation greenhouse in 2008. No fungicides or pesticides were sprayed in this greenhouse. We used one self-rooted vine per genotype, and each vine was 5–7 years old. We monitored disease symptoms on 5–7 berry clusters per clone from 30 to 40 days after full bloom. The disease severity was classified as follows: 0 = no symptoms; 1 = up to 10% of berries infected; 2 = 11–30% of berries infected; 3 = 31–50% of berries infected; 4 = more than 51% of berries infected. The infection index for powder mildew (*IIE*, %) was calculated as follows:

$$IIE = \left(\left[\sum_{i=0}^{4} n_i \times i \right] \Big/ \left[\sum_{i=0}^{4} n_i \times 4 \right] \right) \times 100$$

where i = scale rating (0–4) and n = number of clusters in each scale rating.

Extraction and analysis of resveratrol

The whole flower cluster (0.8–1.2 g) was weighed and homogenized with a homogenizer (POLYTRON PT10-35, Switzerland) in 40 ml methanol. The homogenate was vigorously shaken for 30 min in the dark at 25°C. The mixture was centrifuged at 5000×g for 10 min. The supernatant volume was topped up to 50 ml with methanol and filtered through a 0.45 μm membrane filter for high performance liquid chromatography (HPLC). For green berry extraction, the berries were detached from their clusters, cut in half longitudinally, and deseeded while still frozen. The berry samples (skin + pulp, 2.0–3.0 g) were weighed, homogenized, and prepared for resveratrol analysis using the same methods that were used to prepare flower clusters. HPLC analysis of resveratrol was performed as described by Sato et al. (1997) with some modifications. The HPLC system was a Shimadzu LC-10A gradient liquid chromatograph equipped with a SCL-10A system controller, LC-10AD pumps, a CTO-10A column oven, and a SPD-10A detector. We used a C18 reverse-phase column (CAPCELL PAK Phenyl

UG120, 4.6 × 250 mm, 5 μm particle size, SHISEIDO, Tokyo, Japan). The column was operated at 35°C with a mobile phase flow rate of 1.0 ml min^{-1}. Solvent A was 0.4% phosphoric acid (v/v), and solvent B was 20% solvent A (v/v) and 80% acetonitrile (v/v). The resveratrol peak was detected at 303 nm with a SPD-10A detector (range: 1.0000AUFS; response: 4) using a *trans*-resveratrol standard (Sigma Chemical Co., St. Louis, MO, USA). The gradient elution was initially 0% solvent B, which increased linearly to 30.0% in 20 min. The injection volume was 30–50 μl.

Results

Gray mold experiments

Experiment 1

After UV irradiation, extracts were prepared from flowers and were analyzed by HPLC. The resveratrol peak in the chromatograms of extracts was positively identified by comparison of its retention time with that of the standard. Figure 1 shows typical HPLC profiles of the standard (a), UV-induced resveratrol in Muscat Bailey A (b) and Rosario Bianco (c), and photos of *Botrytis* inoculation in 2007. Muscat Bailey A synthesized more resveratrol and showed few disease symptoms, whereas the susceptible cultivar Rosario Bianco produced very little resveratrol and showed extensive disease symptoms, e.g., aerial mycelia.

Production of resveratrol in response to UV radiation (X) was significantly negatively correlated with the infection index of *Botrytis* (*IIB*, Y) in 2007 ($Y = -3.0758X + 105.83$, $R^2 = 0.8367$, Fig. 2). Muscat Bailey A (27.3 μg g^{-1} FW and $IIB = 2.9\%$) was considered to be highly resistant to *Botrytis*. Moderately *Botrytis*-resistant genotypes with 40–60% *IIB* produced lower levels of resveratrol, ranging from 13.6 μg g^{-1} FW (5512) to 25.3 μg g^{-1} FW (02-10-2). Susceptible genotypes with more than 80% *IIB* synthesized low levels of resveratrol ranging from 3.2 μg g^{-1} FW (Aki Queen) to 6.2 μg g^{-1} FW (Rosario Bianco).

Experiment 2

In 2007, we evaluated resveratrol production in flowers at the early bloom stage in response to UV radiation, and their resistance to *B. cinerea* (Table 2). In all genotypes, UV radiation resulted in resveratrol levels 6- (5512) to 41-times (Yoho) greater than that of their respective controls. Similar to unopened flowers, in opened flowers the UV-induced resveratrol production was strongly negatively correlated with *IIB*. Delaware (resveratrol, 24.7 μg g^{-1} FW; $IIB = 18\%$)

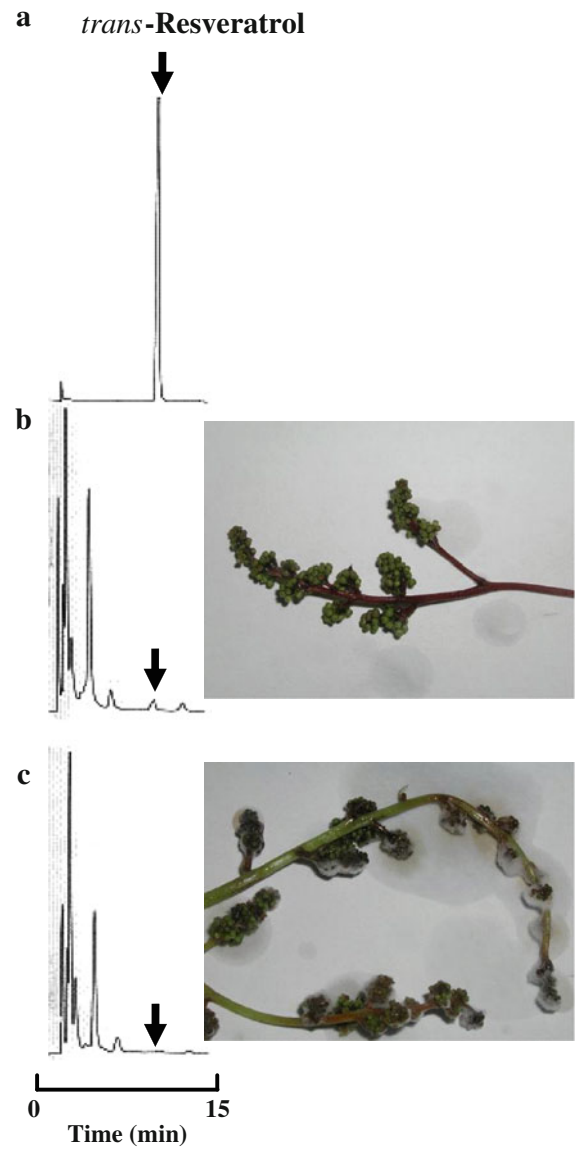


Fig. 1 Typical HPLC profiles of UV-induced resveratrol in grape flower clusters at pre-bloom, and *Botrytis* infection in 2007. **a** standard (*trans*-resveratrol) **b** *Botrytis*-resistant cv. Muscat Bailey A (few disease symptoms) **c** *Botrytis*-susceptible cv. Rosario Bianco (extensive infection with aerial mycelia) *Arrow* indicates *trans*-resveratrol peak

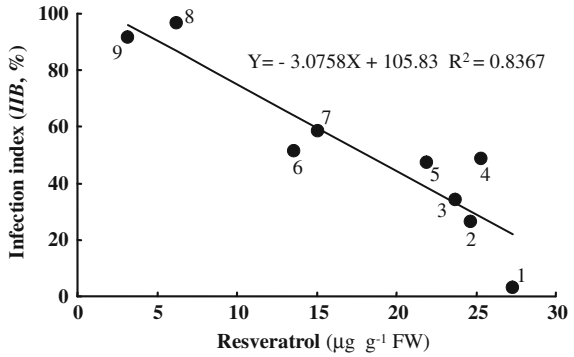


Fig. 2 Relationship between UV-induced resveratrol production and infection index of *Botrytis* (*IIB*) in grape flower clusters at pre-bloom, 2007. *1* Muscat Bailey A, *2* Kyoho, *3* Yoho, *4* 02-10-2 5:02-2-38 6:5512 7: Red Port, *8* Rosario Bianco, *9* Aki Queen

Table 2 Resveratrol production following UV-irradiation and resistance to *Botrytis cinerea* for grape flower clusters at early bloom in 2007

Genotype	Resveratrol content (μg g^{-1} FW)		Infection index of *Botrytis* inoculation (*IIB*) (%)
	UV-irradiated	Control	
5512	2.4a	0.4	96b
Hakata White*	9.5ab	0.0	84b
Kyoho	17.2bc	0.5	68a
Yoho	20.6cd	0.5	24a
Delaware	24.7d	1.1	18a

For each cultivar, experiment consisted of five Petri dishes (replications), each containing three flower clusters on moist filter paper

Mean separation within columns by LSD ($P < 0.05$). Percentage data were arc-sin transformed to obtain a normal distribution

Genotypes are derived from *Vitis vinifera** or interspecific hybrid (*V. labruscana* × *V. vinifera*)

was highly *Botrytis* resistant. Yoho was moderately *Botrytis*-resistant (resveratrol, 20.6 μg g^{-1} FW; 24% *IIB*). Susceptible genotypes with *IIB* values greater than 80% synthesized low levels of resveratrol (2.4 μg g^{-1} FW in 5512; 9.5 μg g^{-1} FW in Hakata White). The UV-induced resveratrol production decreased from the pre-bloom to the early bloom stage in some cultivars, e.g., Kyoho (24.7 at pre-bloom vs. 17.2 at early bloom), Yoho (23.7 vs. 20.6), and 5512 (13.6 vs. 2.4).

Experiment 3

Table 3 shows the UV-induced resveratrol production of 69 grape genotypes at early bloom in 2008. The genotype had a significant effect on UV-induced resveratrol production (ANOVA, $P < 0.05$) and the least significant difference (LSD) among the means of the genotypes was 3.0 μg g^{-1} FW. In general, *vinifera* genotypes produced low levels of resveratrol (<10 μg g^{-1} FW). In American hybrids, resveratrol production varied from 1.1 (Olympia) to 22.8 (Delaware) μg g^{-1} FW. Compared with cultivars, rootstocks derived from American species produced high levels of resveratrol, e.g., Kober 5BB (22.1 μg g^{-1} FW) and Riparia Gloire de Montpellier (26.3 μg g^{-1} FW).

From the results of experiments 1–3, we classified grape genotypes according to their resveratrol production as follows: 37 low-resveratrol (LRG: <10 μg g^{-1} FW), 26 medium-resveratrol (MRG: 10–20 μg g^{-1} FW), and 6 high-resveratrol (HRG: >20 μg g^{-1} FW) genotypes. With respect to gray mold, MRG and HRG cultivars are considered to be field resistant under the general fungicide spray programs used in commercial Japanese viticulture.

Powdery mildew experiments

Experiment 4

In 2007, we determined the changes in resveratrol production in response to UV radiation in green berry extracts of three grape genotypes at 0, 15, and 30 days after full bloom (Table 4). The berries produced significantly ($P < 0.05$) more resveratrol in response to UV radiation over the 30-day period, reaching 20.3 μg g^{-1} FW (5512), 146.0 μg g^{-1} FW (Yoho), and 208.9 μg g^{-1} FW (Campbell Early) by 30 days after full bloom. The amount of resveratrol produced in response to UV radiation differed among the genotypes, ranging from an 8- (Yoho) to 20-times (5512) increase from day 0 to day 30.

Experiment 5

At 30 days after full bloom, soluble solids content of 68 grape genotypes ranged from 2.6 (Red Port) to 4.4 (95-13-2)°Brix, averaging 3.1°Brix among all genotypes. For Riparia Gloire de Montpellier (rootstock), berry sampling was not possible, because this genotype

Table 3 Varietal difference in resveratrol production (in *parentheses*) of grape flowers following UV-irradiation at early bloom in 2008

Resveratrol content (μg g^{-1} FW)	Cultivar or selection
Low (<10)	Olympia (1.1); Palestine* (1.5); Honey Venus (1.8); 02-9-13 (2.0); Bijou Noir* (2.2); Akitsu No. 27 (2.6); Merlot* (2.8); Ruby Seedless* (3.4); Fukuoka No. 13* (3.7); Sunny Dolce* (4.0); 3891 (4.5); Oriental Star (4.8); Rosario Bianco* (4.9); Yatomi Rosa* (4.9); Ruby Okuyama* (5.0); Harmo Noir* (5.0); Steuben (5.0); Muscat Hamburg* (5.3), 02-3-24 (5.8); Festivee (6.4); Portland (6.4); Niagara Rosada (6.6); 97-1-14* (6.7); Muscat of Alexandria* (6.9), Schuyler 4X (7.0); Red Port (7.0); Red Ohanez* (7.1); French Colombard* (7.2); Suiho (7.3); Syrah* (7.4); Rubired (7.7); Shine Muscat (8.6); Neo Muscat* (8.7); Fukuoka No. 14* (8.9); Alden 4X (9.7); 02-3-32 (9.7); Red Port 4X (9.7)
Medium (10–20)	Ohtama Portland (10.9); Gorby (11.3); Fujiminori (11.6); Kai Noir (11.6); 02-7-2 (11.7); Pione (11.9); Sunny Rouge (11.9); 6706* (11.9); Wayne 4X (12.8); Campbell Early (13.2); Alicante Bouschet* (13.6); 02-12-44 (13.6); Cabernet Sauvignon* (13.8); Steuben 4X (15.0); Kyoho (15.4); Fukuoka No. 12 (15.8); 02-2-38 (15.9); Buffalo 4X (16.0); Houman (17.0); Black Beat (17.3); Yoho (17.7); 03-6-1 (17.9); 02-9-4 (18.0); TSA-4 (18.3); 02-10-2 (18.6); Pierce (18.8)
High (>20)	Neo Alicante (20.4); 95-13-2 (21.0); Muscat Bailey A (21.5); Kober 5BB (22.1); Delaware (22.8); Riparia Gloire de Montpellier (26.3)

For each cultivar, experiment consisted of three Petri dishes (replications), each containing three clusters on moist filter paper. ANOVA was used to compare differences among cultivars

Mean separation by LSD ($P < 0.05$) = 3.0 μg g^{-1} FW

Genotypes are derived from *Vitis vinifera** or interspecific hybrid (*V. labruscana* × *V. vinifera*)

Table 4 Resveratrol production in green grape berries following UV-irradiation after full bloom in 2007

Days after full bloom	Resveratrol content (μg g^{-1} FW)		
	Yoho	Campbell early	5512
0	17.4a	15.0a	0.0a
15	45.7a	123.9b	3.2a
30	146.0b	208.9c	20.3b

For each cultivar, experiment consisted of five Petri dishes (replications), each containing three clusters on moist filter paper

Mean separation within columns by LSD ($P < 0.05$)

Genotypes are derived from interspecific hybrid (*V. labruscana* × *V. vinifera*)

has male flowers with nonfunctional pistils, and therefore very low fruit set. Table 5 shows varietal differences in the UV-induced resveratrol production of 68 grape genotypes at 30 days after full bloom in 2008. The genotype had a significant effect (ANOVA, $P < 0.05$) and the LSD among the means of the genotypes was 23.1 μg g^{-1} FW. Portland produced the highest levels of resveratrol (273.1 μg g^{-1} FW), and Muscat Hamburg the lowest (11.6 μg g^{-1} FW). Among the *vinifera* genotypes, resveratrol production was generally low (<50 μg g^{-1} FW), although several cultivars produced higher levels, e.g., Alicante Bouschet (51.3 μg g^{-1} FW), Palestine (76.9 μg g^{-1} FW), and Neo Muscat (85.7 μg g^{-1} FW). Meanwhile, interspecific hybrids generally produced high levels of resveratrol (>50 μg g^{-1} FW), especially North American cultivars, e.g., Delaware (163.9 μg g^{-1} FW), Festivee (170.6 μg g^{-1} FW), and Steuben (245.0 μg g^{-1} FW). However, some American hybrid genotypes produced low levels of resveratrol, e.g., Olympia (17.5 μg g^{-1} FW), Houman (36.1 μg g^{-1} FW), and Kyoho (44.8 μg g^{-1} FW).

Figure 3 shows typical symptoms of green berries naturally infected by *E. necator* in 2008. These vines were grown in an isolation greenhouse and were not sprayed with fungicides or pesticides. Delaware (*IIE* = 22.0%) was considered to be *Erysiphe*-resistant, Rubired was considered to be moderately resistant (*IIE* = 55.6%), and Ruby seedless (*IIE* = 100.0%) was considered to be susceptible. In 2008, UV-induced resveratrol production (X) was significantly ($P < 0.05$) negatively correlated with infection index for powdery mildew (*IIE*, Y) ($Y = -33.995\text{Ln}(X) + 199.43$, $R^2 = 0.9242$, Fig. 4). In general, genotypes with low levels of UV-induced resveratrol (<50 μg g^{-1} FW) were susceptible to powdery mildew, especially *vinifera* genotypes, e.g., Muscat Hamburg, Ruby Okuyama, Red Ohanez, and Ruby Seedless (UV-induced resveratrol levels of 11.6, 17.8, 24.0, and 33.8 μg g^{-1} FW,

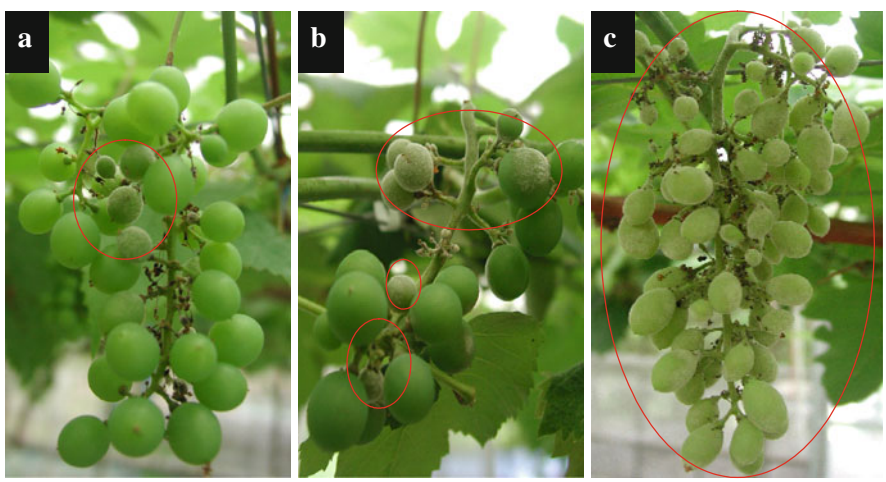
Table 5 Varietal differences in resveratrol production (in *parentheses*) of grape green berries following UV-irradiation at 30 days after full bloom in 2008

Resveratrol content (μg g^{-1} FW)	Cultivar or selection
Low (<50)	Muscat Hamburg* (11.6); 97-1-14* (13.2); Harmo Noir* (16.9); Olympia (17.5); Gorby (17.6); Fukuoka No. 13* (17.7); Ruby Okuyama* (17.8); Sunny Dolce* (18.5); 02-2-38 (18.7); 3891 (19.4); Red Ohanez* (24.0); Ruby Seedless* (33.8); Kai Noir (35.6); Houman (36.1); Syrah* (36.5); 03-6-1 (36.6); Muscat of Alexandria* (38.0); Fukuoka No. 12 (39.2); Yatomi Rosa* (39.4); Rosario Bianco* (40.6); 6706* (40.8); Pione (41.3); Fukuoka No. 14* (43.2); Kyoho (44.8); 02-9-4 (45.7)
Medium (50–100)	Honey Venus (51.0); Alicante Bouschet* (51.3); Merlot* (51.3); French Colombard* (52.4); Suiho (54.8); 02-7-2 (58.5); Rubired (58.9); 02-9-13 (63.3); 02-3-32 (64.6); Oriental Star (66.5); TSA-4 (68.7); 02-12-44 (70.9); 02-3-24 (76.2); Palestine* (76.9); Cabernet Sauvignon* (77.8); Neo Alicante (82.3); Shine Muscat (84.9); Neo Muscat* (85.7), Bijou Noir* (87.1); Akitsu No. 27 (88.8); 02-10-2 (89.8); Alden 4X (90.8); Sunny Rouge (93.9)
High (>100)	Muscat Bailey A (108.6); Black Beat (114.4); Buffalo 4X (132.2); Niagara Rosada (145.3); Yoho (146.0); Pierce (150.9); Schuyler 4X (152.0); Delaware (163.9); Festivee (170.6); Red Port 4X (184.6); Fujiminori (190.4); Steuben 4X (191.0); Wayne 4X (193.9); Kober 5BB (205.4); Campbell Early (218.9); Ohtama Portland (231.1); Red Port (232.4); 95-13-2 (233.6); Steuben (245.0); Portland (273.1)

Each experiment consisted of three Petri dishes (replications), each containing three clusters on moist filter paper. ANOVA was used to compare differences among cultivars

Mean separation by LSD ($P < 0.05$) = 23.1 μg g^{-1} FW

Genotypes are derived from *Vitis vinifera** or interspecific hybrid (*V. labruscana* × *V. vinifera*)



Delaware (*IIE* = 22.0%) Rubired (*IIE* = 55.6%) Ruby Seedless (*IIE* = 100.0%)

Fig. 3 Typical symptoms of grapes naturally infected with *Erysiphe necator* (*IIE*) in isolation greenhouse (no fungicide or pesticide sprays) in 2008. **a** *Erysiphe*-resistant cv. Delaware (partial infection) **b** Moderately *Erysiphe*-resistant cv. Rubired **c** *Erysiphe*-susceptible cv. Ruby Seedless (total infection with mycelia) *Open circles* show areas of infection

respectively). In contrast, genotypes producing higher levels of resveratrol (>100 μg g^{-1} FW) in response to UV radiation were generally resistant to powdery mildew, especially interspecific hybrids such as Buffalo 4X (132.2 μg g^{-1} FW), Steuben 4X (191.0 μg g^{-1} FW), and Campbell Early (218.9 μg g^{-1} FW).

As in the gray mold experiments, the grape genotypes were classified according to their resveratrol production, as follows: 25 low-resveratrol (LRP: <50 μg g^{-1} FW), 23 medium-resveratrol (MRP: 50–100 μg g^{-1} FW), and 20 high-resveratrol (HRP: >100 μg g^{-1} FW) genotypes. LRP genotypes, especially *vinifera*

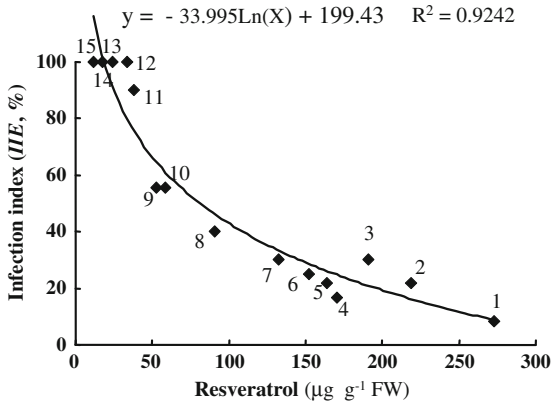


Fig. 4 Relationship between UV-induced resveratrol production and infection index of *Erysiphe* (*IIE*) in grape green berries in 2008. *1* Portland, *2* Campbell Early, *3* Steuben 4X, *4* Festivee, *5* Delaware, *6* Schuyler 4X, *7* Buffalo 4X, *8* Alden 4X, *9* French Colombard, *10* Rubired, *11* Muscat of Alexandria, *12* Ruby Seedless, *13* Red Ohanez, *14* Ruby Okuyama, *15* Muscat Hamburg

cultivars, are likely to be susceptible to powdery mildew in commercial Japanese viticulture.

Discussion

We evaluated the potential of grape flowers and green berries at several developmental stages (pre-bloom until 30 days after full bloom) to produce resveratrol in response to UV treatment. A short UV treatment (15 min) is sufficient to induce resveratrol production in grape genotypes, as shown by other workers (Barlass et al. 1987; Creasy and Coffee 1988; Jeandet et al. 1991). The UV-induced resveratrol production in flowers and berry clusters was positively correlated with resistance to gray mold and powdery mildew. There was also a wide variation in the resveratrol production potential among the genotypes at the early bloom stage and at 30 days after full bloom. Our findings suggest that assaying resveratrol production in response to UV irradiation is effective method to screen for resistance to gray mold at the early bloom stage, and for resistance to powdery mildew at 30 days after full bloom. With respect to sampling of flower clusters, it is easier to select opened flowers (early bloom) than unopened ones (pre-bloom) for screening, because it is easy to judge the stage of flower development in open flowers.

At pre-bloom and early bloom, grape genotypes that produced low levels of resveratrol (<10 μg g^{-1} FW; LRG) in the whole flower in response to UV radiation were susceptible to gray mold. In contrast, those that produced higher levels of resveratrol (>20 μg g^{-1} FW; HRG) were resistant to gray mold. This was observed in both artificial inoculation experiments and in field-grown grapevines. There is little published data about resveratrol content of flower clusters. Creasy and Coffee (1988) analyzed unopened and opened flowers of the interspecific hybrid Chancellor, and found trace quantities of resveratrol ranging from 0.3 (0.02 μg cm^{-2}) to 1.2 (0.07 μg cm^{-2}) μg g^{-1} FW. Keller et al. (2003) showed that the resveratrol levels in *Botrytis*-inoculated *vinifera* Gamay flowers ranged from 8.2 (full bloom) to 14.0 (pre-bloom) μg g^{-1} FW. In addition, they reported that *Botrytis*-inoculated *vinifera* Chardonnay flowers accumulated low levels of resveratrol (1.0–3.0 μg g^{-1} FW). Considering the results of Keller et al. (2003) and those of our study, it appears that *vinifera* cultivars have limited potential for resveratrol production. Adrian et al. (1997) tested the activity of solutions of resveratrol against gray mold, and found that 160 μg ml^{-1} FW inhibited gray mold spore germination, and 60–140 μg ml^{-1} FW inhibited mycelial growth. Compared with their results, however, the gray mold resistant genotypes (HRG) in this study produced much lower levels of resveratrol at early bloom. According to Keller et al. (2003), conidial germination does not guarantee successful penetration and establishment of *Botrytis* infection in grape flowers. Instead, they proposed that the high susceptibility of *vinifera* grape flowers to gray mold may be related not only to their poor capacity for resveratrol synthesis, but also to their constitutively low levels of soluble phenolic compounds (mainly derivatives of quercetin and hydroxyl-cinnamic acid). Gabler et al. (2003) also suggested that disease resistance is a result of the balance between the plant's ability to produce stilbenes (resveratrol) and the ability of the fungus to degrade stilbenes via stilbene oxidase.

The mean soluble solids content among all the genotypes at 30 days after full bloom (the green berries stage) was approximately 3°Brix. This stage coincides with the period of maximum resveratrol production potential in grape berries (Jeandet et al. 1991; Bavaresco et al. 1997). Creasy and Coffee

(1988) pointed out that resveratrol production potential decreased as the soluble solids content increased, therefore, berries could not produce potentially inhibitory concentrations of resveratrol when they reached 5% soluble solids. Gadoury et al. (2001, 2003) reported that ontogenic resistance to powdery mildew in grape berries increased markedly at approximately 3–4 weeks postbloom in an interspecific *V. labruscana* × *vinifera* hybrid and in *vinifera* cultivars. That is, fruit were almost immune to infection before °Brix levels reached 8%. The results of those studies demonstrated that UV-induced resveratrol production of grape berries at 30 days after full bloom is associated with their powdery mildew resistance. In general, American species and their interspecific hybrid genotypes synthesize more resveratrol than *vinifera* genotypes (Bavaresco and Fregoni 2001). In the present study, we determined resveratrol content on a whole berry basis. Therefore, we cannot compare the values obtained in our study with those reported previously, as those values represent resveratrol extracted from fresh skin, the primary site of resveratrol production. As expected, however, the interspecific hybrid genotypes generally accumulated more resveratrol in response to UV treatments than their counterparts. This resulted in the wide variation in UV-induced resveratrol production among grape genotypes in the present study. In a preliminary test, we determined UV-induced resveratrol content of 16 genotypes on the basis of both skin and berry FW. We determined that resveratrol of 1.0 μg g^{-1} skin FW would be equivalent to approximately 16.0 μg g^{-1} berry FW. Takayanagi et al. (2004) reported that the UV-induced resveratrol content of Muscat Bailey A (interspecific hybrid), Chardonnay (*vinifera*), and Koshu (*vinifera*) at 30 days after full bloom was 119.4 μg g^{-1} berry FW (1910 μg g^{-1} skin FW), 79.4 μg g^{-1} berry FW (1270 μg g^{-1} skin FW), and 78.8 μg g^{-1} berry FW (1260 μg g^{-1} skin FW), respectively. Our results are similar to their reports.

When grapevines were cultivated in a greenhouse with no spray program, those that produced low levels of resveratrol (<50 μg g^{-1} FW) in response to UV radiation were more susceptible to powdery mildew infection, particularly the v*inifera* genotypes. In contrast, interspecific hybrid genotypes frequently showed higher resistance to powdery mildew and high UV-induced resveratrol production (>100 μg g^{-1} FW). As is the case in *Botrytis*-resistance, the resistance of grape berries to *E. necator* represents a combination of many attributes, and cannot be attributed to a single resistance factor, i.e., resveratrol production. Previous studies have reported that *Botrytis* infection of grape berries also depends on the number and thickness of epidermal and hypodermal cell layers, the thickness of the cuticle, and the amount of cuticular wax (Dubos et al. 1993; Gabler et al. 2003). For example, highly resistant interspecific hybrid cultivars (e.g., Niagara, Niabell, Fredonia) had few or no pores on the berry surface. The varietal differences in *Erysiphe*-resistance observed in the present study may be attributed, as least in part, to such differences in the anatomy of grape berries. In addition, stilbene synthesis in grapes depends on various viticultural factors such as the cultivar, the environment, and cultural practices. Bavaresco (2003) reported that stilbene concentration in grapes was positively correlated with vineyard elevation.

Some interspecific hybrid genotypes consistently showed low levels of UV-induced resveratrol (<10 μg g^{-1} FW at early bloom, and <50 μg g^{-1} FW at 30 days after full bloom). This is possibly because of recurrent crosses between resistant American interspecific hybrids and susceptible European *vinifera* cultivars to improve fruit quality. Furthermore, resistances of grapevines to gray mold and powdery mildew are polygenic traits (Eibach et al. 1989; Sbaghi et al. 1995). If the potential for resveratrol production is genetically controlled and specific to each grape genotype at the early bloom stage and at 30 days after full bloom, we can establish a simple classification system according to resveratrol production at the two stages, as follows: low and low (type A); low and high (type B); high and low (type C); high and high (type D). For example, Muscat Bailey A is a useful breeding material with high resveratrol production potential (type D). Muscat Bailey A is highly resistant to gray mold infection (Goto and Aono 1981), and accumulates high levels of resveratrol because the genes associated with resveratrol biosynthesis, such as stilbene synthase and phenylalanine ammonia-lyase, are expressed at high levels in this cultivar (Takayanagi et al. 2004). For grape breeding, low levels of UV-induced resveratrol (<10 μg g^{-1} FW) indicate that the genotype is susceptible to gray mold, and levels of less than 50 μg g^{-1} FW indicate susceptibility to powdery mildew.

Acknowledgments We are grateful to Mrs. Harumi Maeda (2007) and Mrs. Chiemi Hirayama (2008), Fukuoka Agricultural Research Center, Fukuoka, for technical assistance with flower and berry sampling and resveratrol extraction.

References

Adrian M, Jeandet P, Veneau J, Weston LA, Bessis R (1997) Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. J Chem Eco 23:1689–1702

Barlass M, Miller RM, Douglas TJ (1987) Development of methods for screening grapevines for resistance to infection by downy mildew. II. Resveratrol production. Am J Enol Vitic 38:65–68

Bavaresco L (2003) Role of viticultural factors on stilbene concentrations of grapes and wine. Drugs Exp Clin Res 29:181–187

Bavaresco L, Eibach R (1987) Investigations on the influence of N fertilizer on resistance to powdery mildew (*Oidium tuckeri*), downy mildew (*Plasmopara viticola*) and on phytoalexin synthesis in different grapevine. Vitis 26:192–200

Bavaresco L, Fregoni C (2001) Physiological role and molecular aspects of grapevine stilbenic compounds. In: Roubelakis-Angelakis KA (ed) Molecular biology and biotechnology of the grapevine. Kluwer Academic Publishers, Dordrecht, pp 153–182

Bavaresco L, Petegolli D, Cantu E, Fregoni M, Chiusa G, Trevisan M (1997) Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. Vitis 36:77–83

Creasy LL, Coffee M (1988) Phytoalexin production potential of grape berries. J Am Soc Hortic Sci 113:230–234

Dercks W, Creasy LL (1989) The significance of stilbene phytoalexins in the plasmopara-grapevine interaction. Physiol Mol Plant Pathol 34:189–202

Dercks W, Creasy LL, Luczka-Bayles CJ (1995) Stilbene phytoalexins and disease resistance in *Vitis*. In: Daniel M, Purkayastha RP (eds) Handbook of phytoalexin metabolism and action. Marcel Dekker Inc., New York, pp 287–315

Dubos B, Prudet S, Charrier L, Le Menn R (1993) Lutte contre le *Botrytis* Difficultés, méthodes actuelles et perspectives. Vigneron Champenois 5:84–93

Eibach R, Diehl H, Alleweldt G (1989) Untersuchungen zur verebung von resistenzeigenschaften bei reben gegen *Oidum tuckeri*, *Plasmopara viticola* und *Botrytis cinerea*. Vitis 28:209–228

Eichhorn KW, Lorenz DH (1977) Phänologosche entwicklungsstadien der rebe. Nachrichtenblatt Deutschrn Pflanzenschutzdienstes (Braunschweig) 29:119–120

Gabler FM, Smilanick JL, Mansour M, Ramming DW, Mackey BE (2003) Correlations of morphological, anatomical, and chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology 93:1263–1273

Gadoury DM, Seem RC, Ficke A, Wilcox WF (2001) The epidemiology of powdery mildew on Concord grapevine. Phytopathology 91:948–955

Gadoury DM, Seem RC, Ficke A, Wilcox WF (2003) Ontogenic resistance to powdery mildew in grape berries. Phytopathology 93:547–555

Goto S, Aono R (1981) Propagation of gray mold of grape, *Botrytis cinerea* on grape berries and leaves. J Insti Enol Vitic Yamanashi Univ 16:1–4

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

Keller M, Viret O, Cole FM (2003) *Botrytis cinerea* infection in grape flowers: defense reaction, latency, and disease expression. Phytopathology 93:316–322

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

Langcake P, McCarthy WV (1979) The relationship of resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. Vitis 18:244–253

Langcake P, Pryce RJ (1977) The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation. Phytochemistry 16:1193–1196

Pearson RC, Goheen AC (1994) Diseases caused by biotic factors. In: Pearson RC, Goheen AC (eds) Compendium of grape diseases. Am Phytopathol Soc, St. Paul, pp 9–59

Pool RM, Creasy LL, Frackelton AS (1981) Resveratrol and the viniferins, their application to screening for disease resistance in grape breeding programs. Vitis 20:136–145

Reisch BI, Pratt C (1996) Grapes. In: Janick J, Moore JN (eds) Fruit breeding: vol 2. Vine and small fruits crops. Wiley, New York, pp 297–369

Sato M, Suzuki Y, Okuda T, Yokotsuka K (1997) Contents of resveratrol, piceid, and their isomers in commercially available wine made from grapes cultivated in Japan. Biosci Biotech Biochem 61:1800–1805

Sbaghi M, Jeandet P, Faivre B, Bessis R, Fournioux JC (1995) Development of methods using phytoalexin (resveratrol) assessment as a selection criterion to screen grapevine in vitro cultures for resistance to gray mould (*Botrytis cinerea*). Euphytica 86:41–47

Stein U, Blaich R (1985) Untersuchungen über stilbenproduktion und botrytisanfälligkeit bei *Vitis*-arten. Vitis 24:75–87

Takayanagi T, Okuda T, Yokotsuka K (2004) Induction of resveratrol biosynthesis in skins of three grape cultivars by ultraviolet irradiation. J Jpn Soc Hort Sci 73:193–199