Accumulation and transportation of resveratrol in grapevines treated by ultraviolet-C irradiation

Junfang Wang[1,2], Chunyan Liu[1], Wei Duan[1], Huifen Xi[1,2], Lijun Wang[1]*, Shaohua Li[1,3]
[1]Key Laboratory of Plant Resources and Beijing Key Laboratory of Grape Science and Enology, Institute of Botany, the Chinese Academy of Sciences, 100093, Beijing, China
[2]Graduate University of Chinese Academy of Sciences, 100049, Beijing, China
[3] Key Laboratory of Plant Germplasm Enhancement and Speciality Agriculture, Wuhan Botanical Garden, the Chinese Academy of Sciences, 430074, Wuhan, China
*E-mail: ljwang@ibcas.ac.cn

Abstract: Resveratrol (3, 5, 4'-trihydroxystilbene) is distributed in almost every part of grapevines. However, little is known if resveratrol may transport among different organs/tissues in grapevines. In this study, the shoots with two or three mature leaves were cut from 'Beifeng' (a hybrid of *Vitis thunbergii* × *V. vinifera*) grapevines. After one leaf of the shoot was treated by ultraviolet C irradiation for 10 minutes, resveratrol (especially *trans*-resveratrol) was synthesized and accumulated in this leaf. Moreover, resveratrol content in the adjacent untreated leaves increased correspondingly. Resveratrol also accumulated in the xylem of the shoot, while the content of total resveratrol in phloem nearly kept unchanged. This indicates that resveratrol in one tissue could be transported to the other tissues through phloem in both apical and basal direction.
[Junfang Wang, Chunyan Liu, Wei Duan, Huifen Xi, Lijun Wang, Shaohua Li. **Accumulation and transportation of resveratrol in grapevines treated by ultraviolet-C irradiation.** *Life Sci J* 2013; 10(2): 2535-2542]. (ISSN: 1097-8135). http://www.lifesciencesite.com 352

Key words: resveratrol; grapevine; transportation; accumulation

1 Introduction

Resveratrol, 3, 5, 4'-trihydroxystilbene, is a polyphenol found in tissues of plants in several families, including Vitaceae and Pinaceae (Gambino, 2006). Interestingly, it has a high medicinal value due to its beneficial effects on human health, such as platelet aggregation inhibition (Bertelli, 1995), cancer chemoprevention (Jang, 1997; Schneider, 2000), antioxidant action (Sanchez-Moreno, 1999), anti-viral activities (Docherty, 1999), anti-inflammatory properties (Martinez, 2000), and cardio-protection (Hung, 2000).

Resveratrol exists as *trans*- or *cis*-resveratrol (monomers), but also as piceids and other polymers. Grapevine is the best-known plant that can biosynthesize and accumulate resveratrol. Resveratrol is distributed in the berry, stem, axillary bud, shoot tip, petiole, root and leaf of grapevines (Wang, 2010). However, resveratrol content varies among different organs and among different tissues of the same organ. For example, resveratrol accumulates in grape berry skins and seeds, while it is hardly detected in the berry flesh. Two picieds with glucosylated forms are mostly located in berry skin (Jeandet, 1991; Roggero, 1995). The content of *trans*-resveratrol in grape skin and seeds varies considerably among different genotypes (Li, 2006). However, to date, most studies have been conducted to investigate the biosynthesis of resveratrol, few have been focused on the transportation of resveratrol among tissues or organs. To reveal if the resveratrol exists transportation among tissues or organs, may help understand the mechanism of higher resveratrol in one organ, and increase resveratrol content in one organ through manipulating the relationship between source and sink.

Ultraviolet C (UV-C) irradiation, fungal infection, ozone, wounds, and some elicitors (methyl jasmonate, salicylic acid) have induced the biosynthesis of resveratrol in berries, leaves and in vitro cultured tissues (Adrian, 2000; Versari, 2001; Chung, 2003; Grimmig, 2002; Belhadj, 2008; Krisa, 1999; Kiselev, 2010). Compared with other abiotic stresses or elicitors, UV-C irradiation is a simple and high efficient treatment for induction of the synthesis of resveratrol in leaves and berries (Cantos, 2001; Bonomelli, 2004). The objective of the present study was to investigate the transportation of resveratrol through using UV-C irradiation to induce resveratrol biosynthesis and accumulation with a high level in grape leaves, focusing on the possibility of exportation and transportation direction.

2 Materials and methods

2.1 Plant materials and treatments

Grapevines of 'Beifeng' (a hybrid of *V. thunbergii* × *V. vinifera*) were used in this study, growing in the experimental vineyard of the Institute of Botany, the Chinese Academy of Sciences, in Beijing, China. Vines were planted in the spring of 2003 and received the same viticultural management practices including irrigation, fertilization, pruning and disease control. Uniform shoots with healthy leaves were harvested in late May for the experiment in 2010. The

treatments were as follows (**Figure 1**): (1) $O_{control}$: two leaves on the opposite sides of the shoot without UV-C irradiation; (2) O_{upper}: two leaves on the opposite sides of the shoot, the upper leaf was treated with UV-C irradiation; (3) O_{lower}: two leaves on the opposite sides of the shoot, the lower leaf treated with UV-C irradiation; (4) $S_{control}$: two leaves on the same sides of the shoot without UV-C irradiation; (5) S_{upper}: two leaves on the same side of the shoot, the upper leaf treated with UV-C irradiation; (6) S_{lower}: two leaves on the same side of the shoot, the lower leaf treated with UV-C irradiation; (7) $OS_{control}$: three leaves were on the shoot, without UV-C irradiation treatment; (8) OS_{middle}: three leaves were on the shoot, the middle leaf treated with UV-C irradiation.

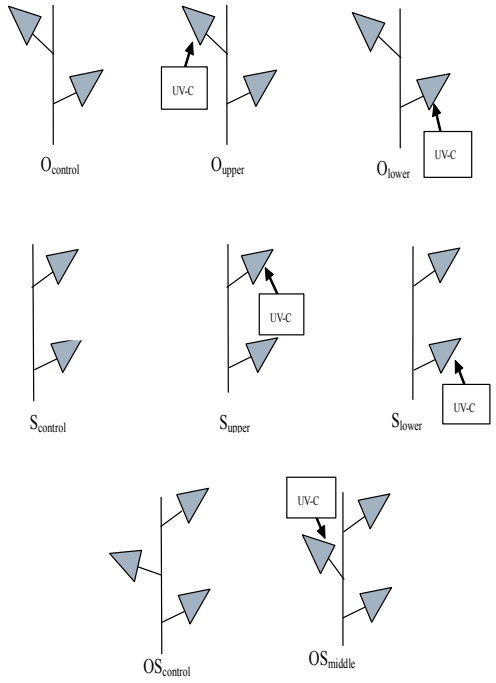

Figure 1. Schematic of the different treatments

Each shoot had two or three leaves. The upper side of an upper, lower or middle leaf of the shoot was exposed for 10 min to 6 $W \cdot m^{-2}$ UV-C irradiation provided by a UV-C lamp. The shoot except for the treated leaf was covered by black reflective cloth (under the cloth, UV-C intensity is 0 $W \cdot m^{-2}$).

$O_{control}$, O_{upper} and O_{lower} indicate that the two leaves are on opposite sides of shoot. $O_{control}$: no UV-C irradiation; O_{upper}: the upper leaf was irradiated by UV-C; O_{lower}: the lower leaf was irradiated by UV-C;

$S_{control}$, S_{upper} and S_{lower} indicate that the two leaves are at the same side of shoot. S_{upper}: no UV-C irradiation upper leaf was irradiated by UV-C; S_{lower}: The lower leaf was irradiated by UV-C;

$OS_{control}$: The three leaves were not UV-C irradiated; OS_{upper}: The middle leaf was UV-C irradiated.

2.2 Method of UV-C irradiation

As shown in Figure 1, the upper, lower or middle leaf of the shoot was exposed for 10 min to 6$W \cdot m^{-2}$ UV-C irradiation provided by a UV-C lamp (Model ZW30S26W, Beijing Lighting Research Institute, China). The shoot except for the treated leaf was covered by black reflective cloth for prevented UV-C light (Under the cloth, UV-C intensity is 0$W \cdot m^{-2}$), and held in a dark room. After the treatment, the basal end of all shoots was immediately inserted into a container with water in a dark growth chamber at 25°C and relative humidity of 60~80%. The shoots were gently sprayed with tap water every three hours throughout the experiment in order to prevent wilting. Shoots with two leaves were sampled at 0 (sampled immediately after treated), 1, 6, 12, 24, 48 and 72 h after UV-C irradiation treated, and sampled the controls also at the same time, all shoots were divided into four parts including upper leaf, lower leaf, and xylem and phloem of the shoot between two leaves. Shoots with three leaves were sampled at 0, 6, 24, 48 and 72 h after UV-C irradiation treated, and sampled the controls also at the corresponding time, the upper, the middle and the lower leaves were used. Then, all samples were frozen in liquid nitrogen, lyophilized, and stored at -40°C for resveratrol measurement.

2.3 Extraction of resveratrol

The lyophilized materials were ground to powder in liquid nitrogen using a grinding machine (A11-b- s25, IKA, China). The resveratrol was extracted according to the methods described by Liu (2013). Each sample was extracted for 24 h in methanol and ethyl acetate (1/1, v/v) (1000 mg samples per 10mL of organic solvent) at 25°C in darkness according to the method of our own laboratory. The suspension was centrifuged at 10,000 × g for 10 min. The supernatant liquid was separated and the resulting residue was extracted another time with 3 ml methanol and ethyl acetate (1/1, v/v). The organic solvent supernatant phases were combined and vacuum-dried with a rotary evaporator (N-1001D-WD, EYELA, Tokyo Rikakikai, Japan) at 40°C. The dried samples were then re-dissolved in 2 mL of pure methanol and stored at -40°C for resveratrol analysis.

2.4 Measurement of resveratrol

Each liquid sample was filtered through a 0.22μm PTFE membrane filter, then the analysis of resveratrol were carried out on a Dionex P680 HPLC system (Dionex Corporation, CA, USA) with a Dionex PDA-100 detector. Separation was achieved using a reverse-phase C18 column of Atlantis® T3 (4.6 mm ×250 mm, 5.0 μm particle size, Waters, USA) and a guard column (Atlantis T3, 4.6 mm× 20 mm, 5.0 μm cartridge, Waters, USA) maintained at 30°C with a Dionex TCC-100 thermostatted column compartment, and the injection volume was 10 μL. Separation was

performed at a flow rate of 1.0 mL·min^{-1} with the mobile phase consisting of H_2O (A) and acetonitrile (B), according to Liu (2013). The solvent gradient was as follows: 0-5 min from 10% to 17% solvent B; 5-12 min from 17% to 18% solvent B; 12-22 min from 18% to 22% solvent B; 22-30 min from 22% to 33% solvent B; 30-45 min from 33% to 38% solvent B; 45-50 min from 38% to 80% solvent B; 50-53 min from 80% to 10% solvent B; 53-60 min 10% solvent B. For fluorimetric detection, the maximum absorption wavelength of two *trans*-isomers (*trans*-resveratrol and *trans*-piceid) was 306nm, and two *cis*-isomers (*cis*-resveratrol and *cis*-piceid) was 288 nm. Three replicates of each sample were scanned by whole wavelength (from 240 to 600nm) (**Figure 2**).

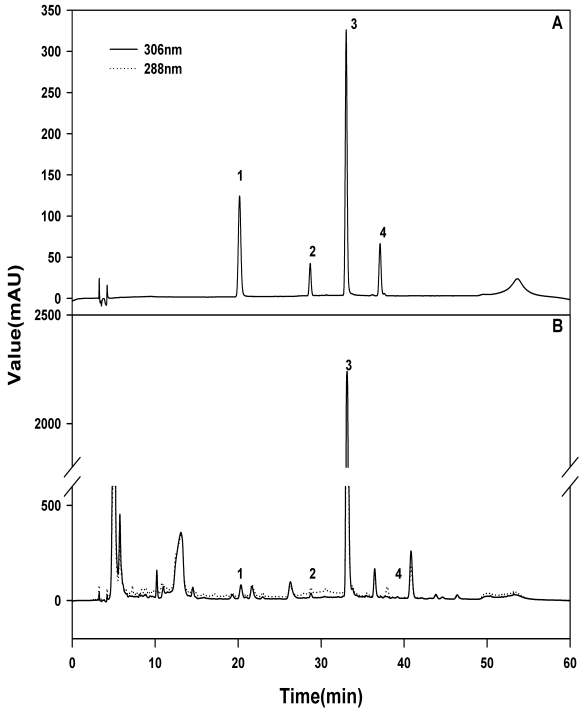

Figure 2. HPLC chromatogram of standards (A), 'Beifeng' leaves (B) at 306 nm (solid line) and 288 nm (dotted line)

The numbers 1, 2, 3 and 4 in the chromatogram indicate *trans*-piceid, *cis*-piceid, *trans*-resveratrol and *cis*-resveratrol, respectively. *Trans*-picied and *trans*-resveratrol were detected at 306nm, and *cis*-picied and *cis*-resveratrol at 288 nm.

Trans-resveratrol and *trans*-piceid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and the Chinese Standards Research Institute, respectively. The mixed solution of the two *trans*-isomers (*trans*-resveratrol and *trans*-piceid) standards were partly transferred to the two *cis*-isomers (*cis*-resveratrol and *cis*-piceid) after UV-C irradiation at 6 W·m^{-2} and a distance of 30 cm for 30 min. Conversion coefficients were computed from the two *trans*-isomers, respectively, and standard curves of the four isomers were made. The total content of resveratrol was obtained by the sum of the four isomers.

2.5 Graphs and data analysis

The content of each compound was plotted over sampling periods from three replications, and experimental data was subjected to analysis of variance using the SPSS 13.0 program (SPSS, USA.). Means were separated by independent-samples T-Test at $P < 0.05$ and $P < 0.01$. All figures were produced by SigmaPlot 10.0.

3 Results

3.1 Resveratrol content in the mesophyll of leaves treated by UV-C irradiation and adjacent untreated leaves

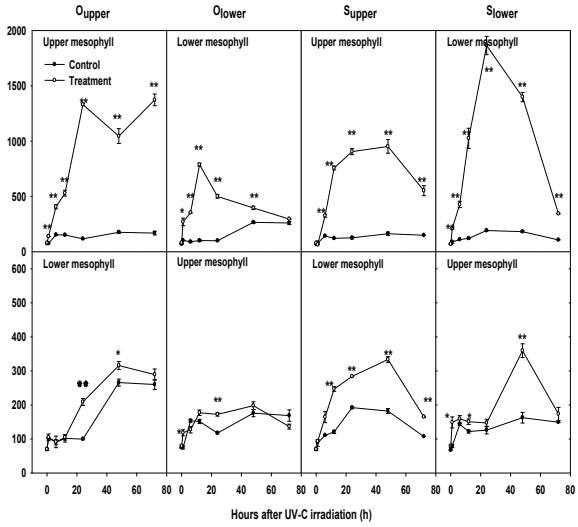

Figure 3. Total resveratrol content of the leaf mesophyll of shoots with two leaves

The first row presents results for control and treated leaves, and the second row presents results for control and corresponding untreated leaves. * indicates difference at P<0.05 between control and treatment, ** indicates difference at P<0.01 between control and treatment.

As shown in **Figure 3**, for the shoot with two leaves, whether the upper or lower leaves were treated by UV-C irradiation, and whether the two leaves were on the opposite or same side of the shoot, the total resveratrol of treated mesophyll increased significantly

in most samples by 100 fold. Notably, when the total resveratrol in treated mesophyll increased, that in untreated mesophyll also increased correspondingly, especially when the two leaves were on the same side of the shoot. The change trend of *trans*-resveratrol content in the mesophyll was similar to that of total resveratrol (**Figure 4**). For the shoot with three leaves, the total resveratrol in the mesophyll of the middle leaf increased significantly after UV-C irradiation, and those in the mesophyll of upper and the lower leaves also increased correspondingly (**Figure 5**). However, the incremental change in the lower leaves was more than in the upper leaves.

3.2 Resveratrol content changed in the phloem and xylem

It was shown in Figure 6 that the total resveratrol in phloem was almost unchanged in all the treatments compared with the controls for shoot with two leaves. Although the total resveratrol in xylem was relatively high before UV-C irradiation, almost 18.82 $\mu g \cdot g^{-1}$ FW (fresh weight), the total resveratrol content in the xylem still increased significantly whether the leaf treated by UV-C irradiation was on the opposite side or the same side of the shoot. Moreover, when the upper leaf was treated by UV-C irradiation (Figure 6 O_{upper} and S_{upper}), the total resveratrol in the corresponding xylem accumulated more. And when the upper leaf on the opposite side was treated by UV-C irradiation (Figure 6 O_{upper}), resveratrol in the corresponding xylem accumulated more and reached a maximum at 48 h after UV-C irradiation. While when the upper leaf on the same side was treated (Figure 6 S_{upper}), resveratrol in the corresponding xylem reached a maximum at 12 h after treatment. However, whether UV-C irradiation treated the lower leaf on the opposite or same side of the shoot (Figure 6 O_{lower} and S_{lower}), resveratrol content in the corresponding xylem increased continuously, but the increment was less.

4 Discussion

4.1 Resveratrol accumulated in mesophyll treated by UV-C irradiation

A study showed that the light feeling spectrum of the key enzyme (resveratrol synthesis) of resveratrol synthesis belongs to the UV-C area (Langcake, 1977). Under the natural condition, UV-C irradiation is almost absorbed by the ozone layer. Therefore, in general, resveratrol content in most grape germplasms was lower. So people may use artificial UV-C irradiation to get more resveratrol. Moreover, some authors have reported that the biosynthesis of *trans*-resveratrol in grape leaves could be induced by UV-C irradiation (Langcake, 1976; Fritzemeier, 1981; Jeandet, 1991; Jeandet, 1992; Bais, 2000; Cantos, 2001; Cantos, 2002; Wang, 2010; Tang, 2011). In this study, as shown in Figure 3 and 5 the total content of resveratrol in mesophyll increased over 100 fold after treated by UV-C irradiation,and the content of *trans*-resveratrol also increased significantly (Figure 4 and 5).

4.2 Resveratrol might be exported from biosynthesize sites and transported bi-directionally through phloem

Though grape leaves and berries could synthesize resveratrol, it was unclear whether resveratrol could be exported from its site of biosynthesis, and if the high resveratrol level in the skin of berries may came from the leaves. In our study, we try to research the problem on the shoot with two leaves or three leaves through UV-C induced high resveratrol biosynthesis. When the total resveratrol in treated leaf (mesophyll) increased after UV-C irradiation, the content of total resveratrol in adjacent untreated leaf increased correspondingly no matter that the untreated leaf was above or below the treated leaf (Figure 3 and 5). Especially, when the two leaves were on the same side of the shoot, the resveratrol content in adjacent untreated leaf increased more. Moreover, when the untreated leaf was below the treated leaf, the resveratrol content in the untreated leaf increased more. The total resveratrol in the mesophyll of upper and lower leaves increased after the middle leaf was treated by UV-C irradiation (Figure 5). These results suggested that resveratrol in grapevines could be exported from a leaf and transported to the other parts in both basal and apical directions, while the main transportation approach was basipetal and ipsilateral. In general, the *trans*-resveratrol content was low in most grapevine genotypes (Li, 2006). After UV-C treatment, *trans*-resveratrol content increased significantly in treated and untreated leaves (Figure 4 and 5). This result indicated that *trans*-resveratrol might be the transportation form for resveratrol in grapevines.

4.3 Phloem was a transportation pathway of resveratrol, while xylem was an accumulation tissue of resveratrol

Phloem had been considered as a pathway of long distance transportion of carbohydrate and nutrient reallocation (Oparka, 1999). In our work, the content of resveratrol in the shoot phloem reached 18.2 $\mu g \cdot g^{-1}$ FW before UV-C irradiation, but was nearly unchanged after UV-C irradiation. Therefore, for revseratrol, the phloem might just be a transport pathway like for carbohydrate instead of an accumulation tissue.

The content of total resveratrol in shoot xylem increased significantly after leaves were treated by UV-C irradiation (Figure 6). The increased resveratrol of xylem should be from the treated leaves. Wang (2010) showed that resveratrol was distributed among different tissues/organs subject to stresses. Others have thought that secondary metabolites and host defense compounds could be translocated from an infected woody tissue to other parts (Bruno, 2006; Bruno, 2007). Bruno (2006) reported that the xylem sap accumulated phenolics and that the content might change due to fungal infection. As a result, we could conclude that

xylem was an important tissue of resveratrol accumulation. Besides, some researches proved that resveratrol might combine with other hydrophilic substances to dissolve in water (Dixon, 1999). Adrian (1997) noted that the two piceids were likely a soluble form of resveratrol in the cell, owing to the fact that hydroxystilbenes such as *trans*-resveratrol were scarcely water soluble. It had previously been suggested that *trans*-resveratrol was synthesized in the free form then rapidly glycosylated to *trans*-piceid (Jeandet, 1997), which occurred and accumulated in xylem with the transport of water. And some studies had shown that stilbenes exist as constitutive compounds in the woody parts of Vitaceae (Bavaresco, 1997), to protect wood decay (Hart, 1979) and provide antifungal activity (Jayasinghe, 2004). They may also occur in the heartwood of many plants in the Pinaceae (Schultz, 1990; Zhao, 2005; Shibutani, 2004; Harju, 2009) and Moraceae (Royer, 2010). Other components, such as flavonoids (Han, 2006; Lee, 2009; Jayasinghe, 2008), xanthones (Jayasinghe, 2008), phenolics and lignin (Gierlinger, 2004; Harju, 2003), hemicellulose and alpha-cellulose (Harju, 2003), and diterpenoids (Zhao, 2005) also exist in the heartwood, all helping to prevent root decay, diseases and insect attack to improve wood durability. So there might be genetic differences that of xylem could accumulate resveratrol.

In addition, the content of resveratrol in xylem increased more when upper leaves were irradiated (Figure 6 O_{upper} and S_{upper}). It was possible that resveratrol was exported from leaves to xylem according to plant biology polarity. The content of total resveratrol increased and accumulated more in the xylem of the shoots with two leaves on opposite sides and the upper leaf was treated (Figure 6 O_{upper}). On the other hand, the resveratrol in the treated leaf on the same side of the shoot may have transported more to the non-treated leaf than the treated leaf on the opposite side (Figure 3).

5 Conclusion

Based on the experiments on the shoots with two or three mature leaves, we can summary that resveratrol in one tissue could be transported to the other tissues through phloem in both apical and basal direction in grapevines.

Acknowledgements

This work was financially supported by Natural Science Foundation of China (Grant No. 31171918) and and Beijing Municipal Science and Technology Plan Project (No. Z121100008512003)).

***Corresponding author:**
Lijun Wang*,
Key Laboratory of Plant Resources and Beijing Key Laboratory of Grape Science and Enology, Institute of Botany, the Chinese Academy of Sciences, 100093, Beijing, China.
E-mail: ljwang@ibcas.ac.cn

References:

[1] Gambino G, Bondaz J, & Gribaudo I. Detection and elimination of viruses in callus, somatic embryos and regenerated plantlets of grapevine. Eur J Plant Pathol 2006; 114(4): 397-404.
[2] Bertelli AAE, Giovannini L, Giannessi D, Migliori M, Bernini W, Fregoni M, & Bertelli A. Antiplatelet activity of synthetic and natural resveratrol in red wine. Int J Tissue React-Ex Clin Asp 1995; 17(1): 1-3.
[3] Jang MS, Cai EN, Udeani GO, Slowing KV, Thomas CF, Beecher CWW, Fong HHS, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, & Pezzuto JM. Cance chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997; 275(5297): 218-220.
[4] Schneider Y, Vincent F, Duranton B, Badolo L, Gosse F, Bergmann C, Seiler N, & Raul F. Anti-prolerative efffect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. Cancer Lett 2000; 158(1): 85-91.
[5] Sanchez-Moreno C, Larrauri JA, & Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int 1999; 32(6): 407-412.
[6] Docherty JJ, Fu MMH, Stiffler BS, Limperos RJ, Pokabla CM, & DeLucia AL. Resveratrol inhibition of herpes simplex virus replication. Antiviral Res 1999; 43(3): 145-155.
[7] Martinez J, & Moreno JJ. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. Biochem Pharmacol 2000; 59(7): 865-870.
[8] Hung LM, Chen JK, Huang SS, Lee RS, & Su MJ. Cardioprotective effect of reveratrol, ca natural antioxidant derived from grapes. Cardiovasc Res 2000; 47(3): 549-555.
[9] Wang W, Tang K, Yang HR, Wen PF, Zhang P, Wang HL, & Huang WD. Distribution of resveratrol and stilbene synthase in young grape plants (*Vitis vinifera* L. cv. Cabernet Sauvignon) and the effect of UV-C on its accumulation. Plant Physiol Biochem 2010; 48(2-3): 142-152.
[10] Jeandet P, Bessis R, & Gautheron B. The production of resveratrol (3, 5, 4'-trihydroxystilb-ene) by grape berries in different developmental stages. Am J Enol Vitic 1991; 42(1): 41-46.
[11] Roggero JP, & 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(5): 411-422.
[12] Li XD, Wu BH, Wang LJ, and Li SH. Extractable amounts of trans-resveratrol in seed and berry skin in *Vitis* evaluated at the germplasm level. J Agric Food Chem 2006; 54(23): 8804-8811.
[13] Adrian M, Jeandet P, Douillet-Breuil AC, Tesson L, & Bessis R. Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. J Agric Food Chem 2000; 48(12) 6103-6105.
[14] Versari A, Parpinello GP, Tornielli GB, Ferrarini R, & Giulivo C. Stilbene compounds and stilbene synthase expression during ripening, wilting, and UV treatment in grape cv. Corvina. J Agric Food Chem 2001; 49(11): 5531-5536.
[15] Chung LM, Park MR, Chun JC, & Yun SJ. Resveratrol accumulation and resveratrol synthase gene expression in response to abiotic stresses and hormones in peanut plants. Plant Sci 2003; 164(1): 103-109.
[16] Grimmig B, Gonzalez-Perez MN, Welzl G, Penuelas J, Schubert R, Hain R, Heidenreich B, Betz C, Langebartels C, Ernst D, & Sandermann H. Ethylene- and ozone-induced regulation of a grapevine resveratrol synthase gene: different responsive promoter regions. Plant Physiol Biochem 2002; 40(10): 865-870.
[17] Belhadj A, Telef N, Saigne C, Cluzet S, Barrieu F, Hamdi S, & Merillon JM. Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. Plant Physiol Biochem 2008; 46(4): 493-499.
[18] Krisa S, Larronde F, Budzinski H, Decendit A, Deffieux G, & Merillon JM. Stilbene production by *Vitis vinifera* cell suspension cultures: Methyl jasmonate induction and C-13 biolabeling. J Na Prod 1999; 62(12): 1688-1690.
[19] Kiselev KV, Dubrovina AS, Isaeva GA, & Zhuravlev YN. The effect of salicylic acid on phenylalanine ammonia-lyase and stilbene synthase gene expression in *Vitis amurensis* cell culture. Russ. J Plant Physiol 2010; 57(3): 415-421.
[20] Cantos E, Espín JC, & Tomás-Barberán FA. Postharvest induction modeling method using UV irradiation pulses for obtaining resveratrol-enriched table grapes: A new "functional" fruit?. J Agric Food Chem 2001; 49(10): 5052-5058.
[21] Bonomelli A, Mercier L, Franchel J, Baillieul F, Benizri E, & Mauro MC. Response of Grapevine defenses to UV-C exposure. Am J Enol Vitic 2004; 55(1): 51-59.
[22] Liu CY, Wang LJ, Wang JF, Wu BH, Liu W, Fan PG, Liang ZC, & Li SH. Resveratrols in Vitis berry skins and leaves: their extraction and analysis by HPLC. Food Chem 2013; 136(2): 643-649.
[23] Langcake P, & Pryce RJ. Production of resveratrol and viniferins by grapevines in response to UV irradiation. Phytochem 1977; 16(8): 1193-1196.
[24] Langcake P, & Pryce RJ. Production of resveratrol by vitis-vinifera and other members of vitaceae as a response to infection or injury. Physiol Plant Pathol 1976; 9(1): 77-86.
[25] Fritzemeier KH, & Kindl H. Coordinate induction by UV-light of stilbene synthase, phenylalanine ammonia-lyase and cinnamate 4-hydroxylase in leaves of vitaceae. Planta 1981; 151(1): 48-52.
[26] Jeandet P, Sbaghi M, & Bessis R. The use of phytoalexin induction and of invitro methods as a tool for screening grapevines for resistance to botrytis-cinerea. Wageningen Pudoc 1992; 109-112.
[27] Bais AJ, Murphy PJ, & Dry IB. The molecular regulation of stilbene phytoalexin biosynthesis in Vitis vinifera during grape berry development. Aus J Plant Physiol 2000; 27(5): 425-433.
[28] Cantos E, Espin JC, & Tomas-Barberan FA. Postharvest stilbene-enrichment of red and white table grape varieties using UV-C irradiation pulses. J Agric Food Chem 2002; 50(22): 6322-6329.
[29] Tang K, Fang F, Yang HR, & Huang WD. Effect of UV-C Irradiation on Stilbene Synthase Localization in Young Grape Plants. *Russ* J Plant Physiol 2011; 58(4): 603-614.
[30] Oparka KJ, & Turgeon R. Sieve elements and companion cells-Traffic control centers of the phloem. Plant Cell 1999; 11(4): 739-750.
[31] Bruno G, & Sparapano L. Effects of three esca-associated fungi on *Vitis vinifera* L.: II. Characterization of biomolecules in xylem sap and leaves of healthy and diseased vines. Physiol Mol Plant Pathol 2006; 69(4-6): 195-208.
[32] Bruno G, & Sparapano L. Effects of three esca-associated fungi on *Vitis vinifera* L.: V. Changes in the chemical and biological profile of xylem sap from diseased cv. Sangiovese vines. Physiol Mol Plant Pathol 2007; 71(4-6) 210-229.
[33] Dixon RA, & Steele LC. Flavonoids and isoflavonoids - a gold mine for metabolic engineering. Trends Plant Sci 1999; 4(10): 394-400.
[34] Adrian M, Jeandet P, Veneau J, Weston LA, & Bessis R. Biological activity of resveratrol, a stilbenic compound from grapevines, against Botrytis cinerea, the causal agent for gray mold. J Chem Ecol 1997; 23(7): 1689-1702.
[35] Jeandet P, Breuil AC, Adrian M, Weston LA, Debord S, Meunier P, Maume G, & Bessis R. HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. Anal

Chem 1997; 69(24): 5172-5177.
[36] Bavaresco L, Cantu E, Fregoni M, & Trevisan M. Constitutive stilbene contents of grapevine cluster stems as potential source of resveratrol in wine. Vitis 1997; 36(3): 115-118.
[37] Hart JH, & Shrimpton DM. Role of stilbenes in resistance of wood to decay. Phytopathol 1979; 69(10): 1138-1143.
[38] Jayasinghe ULB, Puvanendran S, Hara N, & Fujimoto Y. Stilbene derivatives with antifungal and radical scavenging properties from the stem bark of *Artocarpus nobilis*. Nat Prod Re 2004; 18(6): 571-574.
[39] Schultz TP, Hubbard TF, Jin LH, Fisher TH, & Nicholas DD. Role of stilbenes in the natural durability of wood-fungicidal structure-activity -relationships. Phytochem 1990; 29(5): 1501-1507.
[40] Zhao YX, Zhou L, Guo L, Luo XD, & Zhou J. A new diterpenoid and active stilbenes from *Pinus armandii* heartwood. J Asian Nat Prod Res 2005; 7(3): 259-264.
[41] Shibutani S, Samejima M, & Doi S. Effects of stilbenes from bark of *Picea glehnii* (Sieb. et Zucc) and their related compounds against feeding behaviour of *Reticulitermes speratus* (Kolbe). J Wood Sci 2004; 50(5): 439-444.
[42] Harju AM, Venalainen M, Laakso T, & Saranpaa P. Wounding response in xylem of Scots pine seedlings shows wide genetic variation and connection with the constitutive defence of heartwood. Tree Physiol 2009; 29(1):19-25.
[43] Royer M, Herbette G, Eparvier V, Beauchene J, Thibaut B, & Stien D. Secondary metabolites of *Bagassa guianensis* Aubl. wood: A study of the chemotaxonomy of the Moraceae family. Phytochem 2010; 71(14-15): 1708-1713.
[44] Han AR, Kang YJ, Windono T, Lee SK, & Seo EK. Prenylated flavonoids from heartwood of Artocarpus communis with inhibitory activity on lipopolysaccharide-induced nitric oxide production. *J* Nat Prod 2006; 69(4): 719-721.
[45] Lee YJ, Kim S, Lee SJ, Ham I, & Whang WK. Antioxidant activities of new flavonoids from Cudrania tricuspidata root bark. Arch Pharm Res 2009; 32(2) 195-200.
[46] Jayasinghe ULB, Samarakoon TB, Kumarihamy BMM, Hara N, & Fujimoto Y. Four new prenylated flavonoids and xanthones from the root bark of *Artocarpus nobilis*. Fitoterapia 2008; 79(1): 37-41.
[47] Gierlinger N, Jacques D, Schwanninger M, Wimmer R, & Paques LE. Heartwood extractives and lignin content of different larch species (Larix sp.) and relationships to brown-rot decay-resistance. Trees-Struct Funct 2004; 18(2): 230-236.
[48] Harju AM, Venalainen M, Anttonen S, Viitanen H, Kainulainen P, Saranpaa P, & Vapaavuori E. Chemical factors affecting the brown-rot decay resistance of Scots pine heartwood. Trees-Struct Funct 2003; 17(3): 263-268.
6/18/2013

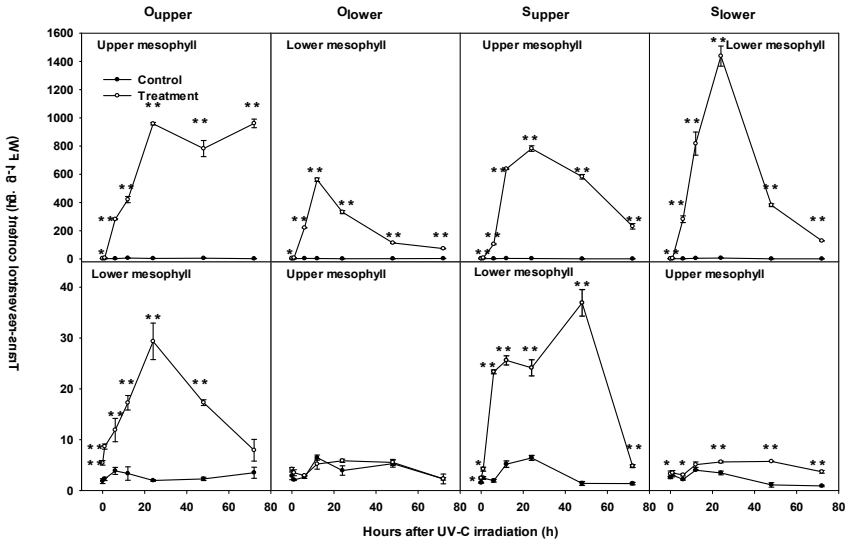

Figure 4. *Trans*-resveratrol content of the leaf mesophyll of shoots with two leaves.

The first row presents results for control and treated leaves, and the second row presents results for control and corresponding untreated leaves. * indicates difference at P<0.05 between control and treatment, ** indicates difference at P<0.01 between control and treatment.

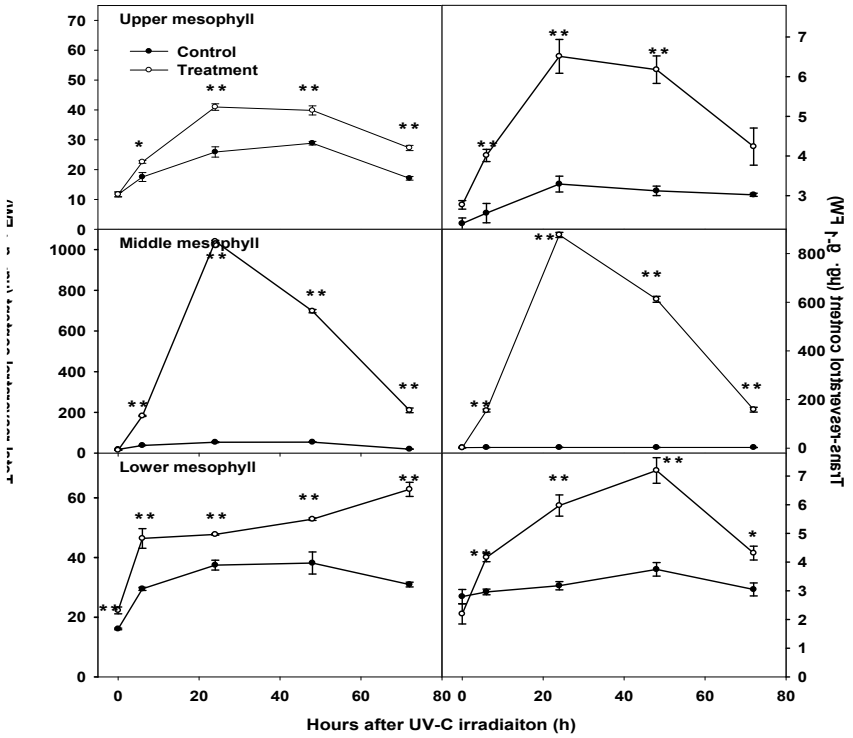

Figure 5. Total resveratrol and *trans*-resveratrol content of leaf mesopyhll of shoots with three leaves

The first row presents results for upper leaves, the second row for treated middle leaves, and the third row for lower leaves. * indicates difference at P<0.05 between control and treatment, ** indicates difference at P<0.01 between control and treatment.

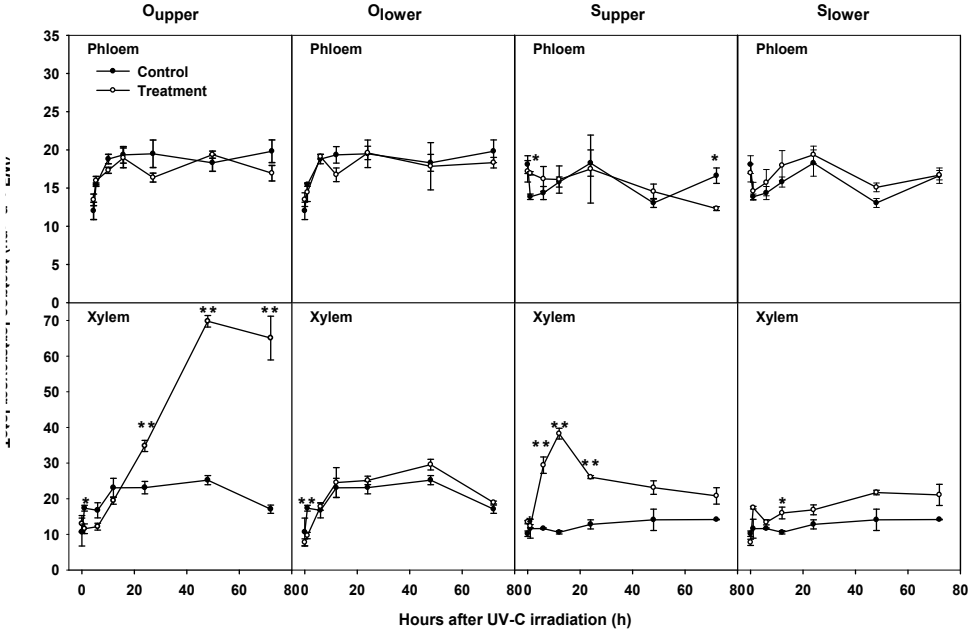

Figure 6. Total resveratrol content of the phloem and xylem between the two leaves of the shoot

* indicates difference at P<0.05 between control and treatment, ** indicates difference at P<0.01 between control and treatment.