Resveratrol Synthesis under Natural Conditions and after Ultraviolet-C Irradiation in Grape Leaves at Different Leaf Developmental Stages

Junfang Wang
Institute of Agro-food Science and Technology/Key Laboratory of Agro-products Processing Technology of Shandong, Shandong Academy of Agricultural Sciences, Jinan 250100, People’s Republic of China; and Beijing Key Laboratory of Grape Science and Enology and Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, People’s Republic of China

Yuxia Sun, Hengzhen Wang, and Xueqiang Guan
Institute of Agro-food Science and Technology/Key Laboratory of Agro-products Processing Technology of Shandong, Shandong Academy of Agricultural Sciences, Jinan 250100, People’s Republic of China

Lijun Wang
Beijing Key Laboratory of Grape Science and Enology and Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, People’s Republic of China

Additional index words. UV-C irradiation, grape leaf, development, resveratrol

Abstract. The biosynthesis and accumulation of resveratrol in grape may be regulated by genetic and environmental factors [i.e., ultraviolet-C (UV-C) irradiation]. However, there is a lack of research on the biosynthesis and accumulation of resveratrol in grape leaves responding to UV-C irradiation at sequential developmental stages. In this study, leaf resveratrol concentration during different developmental stages of three grape cultivars, Jingxiu, Beifeng, and MRH3, under natural conditions, and of ‘Beifeng’ leaves exposed to UV-C irradiation was investigated. Results showed that resveratrol synthesis was related to the developmental stages under natural conditions. In young leaves, resveratrol concentration was low, but it increased continuously during leaf development, mainly as piceid forms. UV-C irradiation greatly stimulated resveratrol synthesis in ‘Beifeng’ leaves, and young leaves at 20 days were most sensitive to the irradiation. The total resveratrol in the skin of UV-C irradiated berries is about 19 times higher than that of the control, mainly in the forms of trans-resveratrol (trans-res)/cis-resveratrol (cis-res). These results provide a basis for increasing resveratrol concentration by artificial means.

Resveratrol (3,4’,5-trihydroxystilbene) is a nonflavonoid polyphenol with a wide range of beneficial biological properties. Since resveratrol was discovered (Takaoka, 1940), it has received considerable attention. Many studies have focused on the bioactive and nutraceutical effects of resveratrol, including its anticancer (Lee et al., 2012; Vislovukh et al., 2013), antioxidant (Csuk et al., 2013), anti-inflammatory (Martinez and Moreno, 2013), and its anticancer (Martinez and Moreno, 2013), antioxidant (Csuk et al., 2013), anti-inflammatory (Martinez and Moreno, 2013), and anti-infectious properties (Dercks and Creasy, 1989; Langcake, 1981; Schmidlin et al., 2008), and abiotic factors, i.e., UV-C irradiation, jasmonic acid, abscisic acid, H2O2, and CaCl2 (Chung et al., 2003; Nicolas et al., 2014; Tassoni et al., 2005; Wang et al., 2013a). UV-C irradiation effectively and quickly induces resveratrol accumulation (Cantos et al., 2003; Crupi et al., 2013; Wang et al., 2015). As the accumulation of resveratrol in berry skin gradually increases during development, the most sensitive stage to UV-C irradiation is 2 weeks before veraison (Wang et al., 2015). Zhang and Liu (2008) reported that resveratrol concentration tripled in young Polygonum cuspidatum leaves after UV-C irradiation, whereas there was no increase in older leaves. Though many studies have focused on resveratrol after UV-C irradiation, it is unknown whether the biosynthesis and accumulation of resveratrol in different cultivars after UV-C irradiation is dependent on the developmental stages of grape leaves.

Herein, we report an investigation on resveratrol change in leaves throughout leaf development in different cultivars, and the sensitivity of resveratrol synthesis and accumulation in different development stages on exposure to UV-C irradiation. This study will provide useful information for the direct use of UV-C irradiation in practice, such as a dose of UV-C irradiation may be applied to leaves at proper development stages to induce resveratrol concentration that may then increase resveratrol transport to and concentration in berries.

Materials and Methods

Plant materials and treatments. Leaves of three cultivars, Jingxiu (Vitis vinifera), Bei-feng [Vitis thunbergii × Muscat Humburg (V. vinifera)], and MRH3 (Vitis monticola × Vitis riparia), were used in this study. All grapevines were grown in the experimental vineyard at the Institute of Botany, Chinese Academy of Sciences, Beijing, China. The vineyard received regular management practices including irrigation, fertilization, pruning, and disease control (NY/T 5088-2002, Ministry of Agriculture of the People’s Republic of China).

Received for publication 30 Dec. 2015. Accepted for publication 24 Apr. 2016.

This work was financially supported by the National Natural Science Foundation of China (Grant no. 31171918).

Corresponding author. E-mail: 18660156607@126.com or ljwang@ibcas.ac.cn.

Fig. 1. Schematic diagram of ultraviolet-C irradiation.
Two experiments were conducted in 2013. Experiment 1 assessed resveratrol accumulation in leaves of the three cultivars throughout leaf development. Experiment 2 assessed resveratrol accumulation in UV-C irradiated leaves.

For experiment 1, healthy leaves were sampled at different developmental stages under natural conditions. The leaves were labeled when the leaves emerged, and healthy leaves were randomly harvested after 5, 10, 20, 30, and 40 d. Three independent replicates of each development stage were sampled, and each replicate consisted of six leaves. After sampling, the leaves were taken to the laboratory immediately, ground into powder in liquid nitrogen, and then stored at −80 °C until analysis.

In experiment 2, leaves of ‘Beifeng’ were sampled at 20, 30, and 40 d after emergence. After sampling, the abaxial sides of 50% of the leaves were irradiated with UV-C (254 nm, Model ZW30S26W; Beijing Lighting Research Institute, China) at a 15-cm distance (power 6 W m⁻²) for 10 min in the dark at 25 °C (Wang et al., 2013a), and the leaf petioles were then inserted into triangular flasks containing double-distilled water (ddH₂O) until sampling. The remaining leaves were taken as control leaves which were also placed in the dark at 25 °C for 10 min, then the leaf petioles were incubated in triangular flasks containing ddH₂O until sampling (Fig. 1). Leaves were then collected at 0, 3, 6, 12, 24, 36, and 48 h after treatment. All the treatments had three independent replicates, and each replicate consisted of six leaves. After sampling, the leaves were ground into powder in liquid nitrogen, and then stored at −80 °C until analysis.

Resveratrol determination. According to the method of Liu et al. (2013), each sample was extracted for 24 h in methanol and ethyl acetate (1/1, v/v; 1000 mg per 10 mL of organic solvent) (Beijing Chemical Works, Beijing, China) at 25 °C in the dark. The suspension was centrifuged at 10,000 g, for 10 min. The supernatant was separated, and the resulting residue was extracted with 3 mL methanol and ethyl acetate (1/1, v/v). The organic solvent phases were pooled and vacuum-dried in a rotary evaporator (N-1001D-WD; EYELA, Tokyo Rikakikai, Japan) at 40 °C. The dried samples were redissolved in 2 mL pure methanol and stored at −40 °C for resveratrol analysis.

Each sample was filtered through a 0.22-μm polytetrafluoroethylene membrane filter (SCAA-204; Anpel, Shanghai, China). Resveratrol was analyzed in a Dionex P680 HPLC system (Dionex Corporation, CA) coupled to a Dionex PDA-100 detector. Separation was achieved using a reverse-phase C18 column of Atlantis® T3 (4.6 × 250 mm, 5.0-μm particle size, Waters, MA) and a guard column (Atlantis T3, 4.6 × 20 mm, 5.0-μm cartridge, Waters) maintained at 30 °C with a Dionex TCC-100 thermostat column. 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 water (solvent A) and acetonitrile (solvent B) (Sigma-Aldrich, St. Louis, MO). The solvent gradient was performed in the following manner: 0–5 min with 10% to 17% solvent B; 5–12 min with 17% to 18% solvent B; 12–22 min with 18% to 22% solvent B; 22–30 min with 22% to 33% solvent B; 30–45 min with 33% to 38% solvent B; 45–50 min with 38% to 80% solvent B; 50–53 min with 80% to 10% solvent B; and 53–60 min with 10% solvent B. For fluorimetric detection, the maximum absorption wavelength of the two trans-isomers (i.e., trans-res and trans-pd) was 306 nm and of the two cis-isomers (i.e., cis-res and cis-pd) was 288 nm. Three replicates of each sample were also scanned from 240 to 600 nm (Fig. 2).

Trans-res and trans-pd standards were purchased from Sigma-Aldrich and the Chinese Standards Research Institute, respectively. The mixed solution of the two trans-
isomer (trans-res and trans-pd) standards were partly converted to the two cis-isomers (cis-res and cis-pd) after UV-C irradiation for 30 min at 6 W m⁻² and a distance of 15 cm (Adrian et al., 2000; Liu et al., 2013). Conversion coefficients were computed from the two trans-isomers; standard curves of the four isomers were generated.

Graphs and data analysis. Graphs of the experimental data were developed using Sigma Plot 12.3 (SPSS Inc., Chicago, IL) for Windows. The concentration of each compound was plotted over sampling periods from three replicates, and experimental data were subjected to analysis of variance using the SPSS 13.0 program (SPSS). Means were separated by paired-sample t test (at $P < 0.05$, $P < 0.01$, and $P < 0.001$).

Results

Changes of resveratrol concentration in leaves throughout development periods. Four resveratrol isomers were detected in leaves

Fig. 4. Total resveratrol and its component concentrations in leaves following ultraviolet-C irradiation at different developmental stages of ‘Beifeng’ [Vitis thunbergii × Muscat Humburg (Vitis vinifera)]. Data are mean of three replicates and their se. The symbols *, **, and *** indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, between control and treatment.
throughout development, including trans-pd, cis-pd, trans-res, and cis-res (Fig. 3). Total resveratrol concentration reflected the accumulation of the four isomers. The pattern of increasing concentration of total resveratrol was similar in the three cultivars, though the concentration differed among them. Total resveratrol concentration remained almost unchanged in young leaves (5 and 10 d) of three cultivars. However, total resveratrol concentration increased rapidly in ‘MRH3’ leaves during leaf development, whereas it increased slowly in ‘Jingxiu’ and ‘Beifeng’ leaves (Fig. 3A). As for the isoforms, the concentration of the two piceids increased continuously until 40 d, and the increasing trend was similar to that for total resveratrol (Fig. 3B and C). Trans-res concentration increased only in ‘MRH3’ leaves, whereas it remained almost unchanged in ‘Jingxiu’ and ‘Beifeng’ leaves (Fig. 3D). However, cis-res concentration was low in the three cultivars, especially in ‘Jingxiu’ leaves (Fig. 3E).

Resveratrol synthesis in leaves exposed to UV-C irradiation. Similar to leaves under natural conditions, the four isomers (trans-pd, cis-pd, trans-res, and cis-res) were detected in UV-C irradiated leaves (Fig. 4). Therefore, total resveratrol concentration reflected the accumulation of the four isomers. Total resveratrol in control leaves was unaffected during the experimental period, but it increased significantly in UV-C irradiated leaves. Levels of trans-pd and cis-pd changed in response to UV-C irradiation, though the increment was small and inconsistent for cis-pd. Trans-res synthesis was quite sensitive to UV-C irradiation, whereas cis-res was less sensitive.

The effect of UV-C irradiation on resveratrol synthesis was highly dependent on leaf developmental stage. Trans-pd and trans-res were sensitive to UV-C irradiation at a young stage, especially trans-res. Consequently, total resveratrol concentration in leaves at 20 d dramatically increased following UV-C irradiation (Figs. 4 and 5). During leaf development, the later increment times of total resveratrol were lower than at earlier stages because synthesis of trans-pd and trans-res were dramatically decreased following UV-C irradiation. In contrast, cis-pd was relatively insensitive to UV-C at young leaf stages and even less sensitive after leaf maturation. During the period after the leaves were cut, trans-res concentration rapidly increased during the first 12 h and 24 h of the first and the last two developmental stages; however, it decreased considerably after 12 or 24 h. Trans-res was the dominant form after UV-C irradiation; therefore, the change in total resveratrol was similar to that for trans-res in leaves at the three developmental stages. Compared with trans-res, trans-pd and cis-pd concentration were considerably lower. However, during the posttreatment period, cis-res concentration remained nearly unchanged during first 12 h, but increased rapidly after 12 h till to 48 h, which showed an opposite tendency with trans-res. Thus, the concentration of cis-res was the dominant form at the later posttreatment periods.

Discussion

Resveratrol levels were related to leaf age and accumulation patterns were dependent on trans-pd and cis-pd under natural conditions. Previous studies had reported total resveratrol concentration was unaffected before veraison, and then constantly increased from veraison to complete maturity under natural conditions (Gatto et al., 2008; Versari et al., 2001; Wang et al., 2015). In this study, increasing total resveratrol concentration in developing leaves was very similar to that in berries. Total resveratrol concentration was relatively lower in young leaves, and increased continuously with the development of leaves. Moreover, its accumulation was mainly dependent on trans-pd and cis-pd (Fig. 3). The results revealed that resveratrol glucosides (trans-pd and cis-pd) were preferentially accumulated in ‘Beifong’ leaves, which was similar to that in berries (Wang et al., 2015). Trans-res concentration was higher in young leaves (5 d old) of ‘Jingxiu’ compared with the other cultivars, but it did not change during development. In leaves of ‘Beifeng’, trans-res concentration was barely detected in young leaves (5, 10, and 20 d old), and only reached about 5 μg g⁻¹ FW in leaves that were 30 and 40 d old. However, trans-res concentration increased considerably in ‘MRH3’ during leaf development (Fig. 3D). These results may be related to differing expression of STS and O-3-GT among the three cultivars (Fang et al., 2014; Holl et al., 2013; Wang et al., 2015). Understanding the roles of STS and O-3-GT in the different cultivars will require further research.

Young leaves were more sensitive to UV-C irradiation than older leaves. Resveratrol synthesis varies with the developmental stages. Previous studies have focused on resveratrol synthesis and accumulation in the skin of grape berries. Jeandet et al. (1991) showed that resveratrol synthesis decreased with berry development. Bas et al. (2000) and Creasy and Coffee (1988) also reported that resveratrol synthesis was most sensitive in berries 5 weeks after anthesis, and resveratrol concentration of UV-irradiated berries was close to zero in ripe fruits. In addition, our previous study throughout the entire period of berry development proved that berries at 55 d after anthesis (DAA) of ‘Beihong’ (V. vinifera × Vitis amurensis) were most sensitive to UV-C irradiation, followed by 40, 70 (veraison), 84, 112, and 126 DAA (maturity) (Wang et al., 2015). Our goal in this study was to determine which developmental stage(s) was most sensitive to UV-C irradiation.

In this study, UV-C irradiation significantly stimulated accumulation of resveratrol in ‘Beifeng’ leaves no matter the development stage (Fig. 4), in agreement with previous studies (Crupi et al., 2013; Liu et al., 2010; Wang et al., 2013a; Xi et al., 2015). Resveratrol increased most in young leaves (20 d old), followed by mature leaves (30 d old), and older leaves (40 d old). Others have found that resveratrol concentration in berries was correlated to that in leaves (Lamuela-raventos et al., 1995), and trans-res concentration was significant in grape stems (Bavaresco et al., 1997). Our studies have demonstrated that the response of resveratrol synthesis to UV-C irradiation was leaf-age dependent, that resveratrol concentration in berry skin was correlated with that in leaves, and that resveratrol was transported in both directions in the phloem in the form of trans-res (Wang et al., 2013; Zheng et al., 2009). On the basis of the results, some methods could be used in moderation to stimulate resveratrol synthesis and accumulation in leaves, thus improving berry resveratrol as a result of the transport.

In the present study, UV-C irradiation induced resveratrol accumulation in ‘Beifeng’ leaves, mainly in the forms of trans-res and cis-res, which was different from that in berry skin (Wang et al., 2013b, 2015). The main form in the earlier periods (less than 24 h after UV-C irradiation) was trans-res,
whereas cis-res was the main form in the later periods (24–48 h after UV-C irradiation). The main reason might be that trans-res was converted to cis-res by isomerases, or converted to piceids by O-3-GT during the posttreatment periods. However, the mechanisms of isomerases and O-3-GT need further study.

Conclusions

Under natural conditions, resveratrol synthesis in leaves was related to leaf development. Resveratrol concentration was low in young leaves and increased with leaf development, primarily in the form of trans/cis-pd. UV-C irradiation significantly stimulated the synthesis of resveratrol, mainly as trans/cis-res. The response of leaves to UV-C irradiation was also related to their developmental stages. Leaves that were 20 d old were the most sensitive to UV-C irradiation. Along with developmental factors, the sensitivity of resveratrol synthesis to UV-C irradiation gradually declined.

Literature Cited

Adrian, M., P. Jeandet, A.C. Douillet-Breuil, L. Tesson, and R. Bessis. 2000. Stilbene content of mature Vitis vinifera berries in response to UV-C elicitation. J. Agr. Food Chem. 48(12): 6103–6105.

Bais, A.J., P.J. Murphy, and I.B. Dry. 2000. The mechanisms of isomerases and O-3-GT need further inverted to piceids by O-3-GT during the

Donnez, D., P. Jeandet, C. Clement, and E. Courot. 2009. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. Trends Biotechnol. 27(12):706–713.

Fang, L.C., Y.L. Hou, L.J. Wang, H.P. Xin, N. Wang, and S.H. Li. 2014. Myb14, a direct activator of STS, is associated with resveratrol content variation in berry skin in two grape cultivars. Plant Cell Rpt. 33(10):1629–1640.

Gatto, P., U. Vrhosek, I. Muth, C. Seràla, C. Romualdi, P. Fontana, D. Pruefer, M. Stefanini, C. Moser, F. Mattivi, and R. Velasco. 2008. Ripening and genotype control stilbene accumulation in healthy grapes. J. Agr. Food Chem. 56(24):11773–11785.

Holl, J., A. Van佐zi, S. Czernzel, C. D’Onofrio, A.R. Walker, T. Rausch, M. Lucchin, P.K. Boss, I.B. Dry, and J. Bogs. 2013. The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis in Vitis vinifera. Plant Cell 25(10):4135–4149.

Jeandet, P., R. Bessis, and B. Gauteron. 1991. The production of resveratrol (3,5,4’-trihydroxy stilbene) by grapeberries in different developmental stages. Amer. J. Enol. Viticult. 42(1): 41–46.

Langcake, P. 1981. Disease resistance of Vitis spp. and the production of the stress metabolites resveratrol, epsilon-viniferin, alpha-viniferin and pterostilbene. Physiol. Plant Pathol. 18(2): 213–226.

Lee, H.S., A.W. Ha, and W.K. Kim. 2012. Effect of resveratrol on the metastasis of 4T1 mouse breast cancer cells in vitro and in vivo. Nutr. Res. Pract. 6(4):294–300.

Li, X.D., B. Wu, L.J. Wang, and S.H. Li. 2006. Extractable amounts of trans-resveratrol in seed and berry skin in Vitis evaluated at the germplasm level. J. Agr. Food Chem. 54(23): 8804–8811.

Liu, C.Y., L.J. Wang, J.F. Wang, B.H. Wu, W. Liu, P.G. Fan, Z.C. Liang, and S.H. Li. 2013. Resveratrol accumulation and synthesis gene expression in response to abiotic stresses and hormones in peanut plants. Plant Sci. 194(1):103–110.

Craeye, J.L., and M. Coffe. 1988. Phytoalexin production potential of grape berries. J. Amer. Soc. Hort. Sci. 113(2):230–234.

Crupi, P., A. Pichieri, T. Basile, and D. Antonacci. 2013. Postharvest stilbenes and flavonoids enrichment of table grape cv Red Globe (Vitis vinifera L.) as affected by interactive UV-C exposure and storage conditions. Food Chem. 141(2):802–808.

Cusk, R., S. Albert, and B. Siewert. 2013. Synthesis and radical scavenging activities of resveratrol analogs. Arch Pharm 346(7):504–510.

Dercks, W. and L.L. Craeye. 1989. The significance of stilbene phytoalexins in the Plasmodiophora viticola-grapevine interaction. Physiol. Mol. Path Plant. 34(3):189–202.

Donnez, D., P. Jeandet, C. Clement, and E. Courot. 2009. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. Trends Biotechnol. 27(12):706–713.

Tadaoka, M.J. 1940. Of the phenolic substances of Veratrum grandiflorum (Veratrum grandiflorum L.) as affected by interactive UV-C irradiation. J. Agr. Food Chem. 48(12): 6103–6105.

Bais, A.J., P.J. Murphy, and I.B. Dry. 2000. The mechanism of isomerases and O-3-GT need further inverted to piceids by O-3-GT during the

Donnez, D., P. Jeandet, C. Clement, and E. Courot. 2009. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. Trends Biotechnol. 27(12):706–713.

Tadaoka, M.J. 1940. Of the phenolic substances of Veratrum grandiflorum (Veratrum grandiflorum L.) as affected by interactive UV-C irradiation. J. Agr. Food Chem. 48(12): 6103–6105.