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Optimising UV-C preharvest light for stilbene synthesis stimulation in table grape: Applications

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ABSTRACT

UV-C treatment has been studied for increasing stilbene in grape and inhibiting the development of spoilage microorganism. In this work, treatment application day (7, 5, 3 and 1 days previous to harvest) and light dose (from 966 to 27,990 J/m^2) have been optimised together with storage conditions (20 °C–80% RH, 4 °C–60% RH and combined) when grapes were treated on plant with UV-C light. *trans*-Resveratrol and ε-viniferin were monitored in grapes after treatments. Maximum *trans*-resveratrol concentration was achieved after 24 h regardless of preharvest application day. It increased between 46 to and 22-folds. A dose lower than 10,000 J/m^2 approximately seemed to increase *trans*-resveratrol. *trans*-Resveratrol was affected not only by the dose but also by how the dose was applied in terms of output power and exposure time. Cold storage keeps stilbene metabolism still until grapes reach a temperature which re-activates their synthesis.
Industrial relevance: Preharvest UV-C light treatment is an interesting stilbene inductor in table grapes on plant and during postharvest storage. Due to the health promoting and anti-pathogen properties of stilbenes, the use of this technology is interesting for both the consumer preferences and organic/viticulture point of view.

1. Introduction

Ultraviolet C light (UV-C) is a germicidal non-ionising radiation, with a wavelength range from 200 to 280 nm. It has been widely studied for increasing bioactive stilbenes in grape and disinfecting (recently reviewed by Tříska & Houška, 2012). Initially, most of UV-C experimental studies were carried out with the goal of inhibiting the development of spoilage. Later, the interest was focused on increasing the production of stilbenes, mainly *trans*-resveratrol in grapes and wine. The majority of them were applied on postharvest grapes (Guerrero, Puertas, Fernández, Palma, & Cantos-Villar, 2010).

When grape berries are illuminated with UV-C, the berries respond to this stress by inducing phytoalexins, mainly stilbenes. Stilbenes are phenols derived from the phenylpropanoid and acetate–malonate pathways expressed in many plant families such as *Vitaceae* (Shen, Wang, & Lou, 2009). Stilbenes, mainly *trans*-resveratrol, have aroused increasing attention lately due to their many health-promoting properties (antioxidant, anticarcinogenic, anti-inflammatory, cardioprotective, and neuroprotective activities, among others) (reviewed by Guerrero, Garcia-Parrilla, Puertas, & Cantos-Villar, 2009). Moreover, their antifungal properties were deeply study in the 90s (Douillet-Breuil, Jeandet, Adrian, & Bessis, 1999).

Although less studied than *trans*-resveratrol, resveratrol oligomers including ε-viniferin (dimeric stilbene) have been shown to have bioactivity such as antioxidant, anticancer, anti-inflammatory, cardiovascular protection, antithrombotic, estrogenic, central nervous system related activity, and modulation of lipid metabolism (Niesen, Hessler, & Seeram, 2013; Xue et al., 2014). ε-Viniferin is also known to display significant anti-pathogenic properties (antibacterial and antifungal), such as activity against downy mildew (*Plasmopara viticola*), grey mould (*Botrytis cinerea*), *Phoma medicaginis*, *Rhizopus stolonifer*, and a broad spectrum of microbes and fungi present during storage (Lambert et al., 2013; Schnee et al., 2013). Its concentration is intimately connected biogenetically with *trans*-resveratrol (Adrian, Jeandet, Douillet-Breuil, Tesson, & Bessis, 2000).

Douillet-Breuil et al. (1999) confirmed the role played by *trans*-resveratrol in the active defence mechanisms of grapevines when UV-C light was used as elicitor in leaves. It was concluded that resistance of *Vitis* genotypes to *B. cinerea* appeared to be strongly correlated to the production of two major phytoalexins, that is, *trans*-resveratrol and ε-viniferin. The ability to produce *trans*-resveratrol and derivatives has been found to be a good marker for grape disease tolerance or resistance

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in vines (Borie, Jeandet, Parize, Bessis, & Adrian, 2004). Thus stimulation of grapevine phytoalexins could be a strategy to limit the use of pesticides in vineyards. Additionally, there is a gathered finding that UV-C light can lead to possible "functional" grapes, within normal conditions of market commercialization, responding to the rising consumer demand to have foods that support and promote health (Barreiro-Hurlé, Colombo, & Cantos-Villar, 2008).

As UV-C light treatment has not been studied on grapes before harvest, the aim of the present work was to study the UV-C light as a preharvest elicitor in table grape Red Globe through the analysis of *trans*-resveratrol and ε-viniferin. Treatment application day and light dose were optimised together with storage conditions. Its application as a technology to both, control microorganism in vineyard and increase health promoting compounds is discussed.

2. Materials and methods

2.1. Reagents

trans-Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) was purchased from Sigma-Aldrich (Steinheim, Germany). ε-Viniferin (5-[(2R,3R)-6-Hydroxy-2-(4-hydroxyphenyl)-4-[(E)-2-(4-hydroxyphenyl)ethenyl]-2,3-dihydro-1-benzofuran-3-yl]benzene-1,3-diol) was obtained from a purified extract kindly provided by the GESVAB group from University of Bordeaux II. Analytical grades of acetic acid, formic acid, methanol and diethyl ether solvent were supplied by Panreac (Barcelona, Spain). Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used in this research.

2.2. Plant and grape material

The experiments were conducted in 2010, 2011 and 2012 harvests on the table grape variety cv Red Globe (*Vitis vinifera* L.) The vineyard is located in a trial site of the IFAPA Centre "Rancho de la Merced", Jerez de la Frontera (SW Spain, long. 06:00:58 W, lat. 36:45:29 N) at 35 m on the sea level and planted in a limestone soil composed of 19% sand, 38.5% clay and 42.5% silt. All vines were planted in 2004 on Selection Oppenheim 4 root-stocks, with a planting density of 1600 vines/ha, and a vine spacing of 2.50 m between rows and 2.50 m within a row.

15 plants were trained in the parral system. Trunk was divided into four shoots, with 8–10 spurs each to aim approximately 1–2 grape clusters/spur. Plants were previously marked and leafless. Cluster thinning was also performed if needed to leave clusters more exposed to light.

Grape ripeness (sugars, total acidity and pH) was monitored to determine the optimum harvest date. 2 grapes were randomly and carefully picked from each bunch (approximately 200 g in total), and peeled using a sharp knife. Grape skins were frozen at −80 °C until extraction was performed.

2.3. Ultraviolet C light system description

The preharvest UV-C chamber comprised a stainless steel tripod extensible from 1.6 m up to 1.8 m. Five hinged stainless steel sheets on the top shaped like a truncated pyramid, being two sides 1100 mm long and 600 mm wide. The other two sides were 600 mm long and 600 mm wide while the lower sheet was 1100 mm long and 300 mm wide. This structure was able to bring down the four sides. Electric lamps were placed in two of the side and bottom sheets. There were 8 lamps: 3 lamps on each side sheet and 2 lamps on the bottom one. Lamps were Philips model TUV SE130WXPT (Spain) with an electric power and UV-C radiation output of 130 W/lamp (total power 1040 W) and 52 W/lamp (416 W), respectively. The 8 electronic ballasts that fed the lights were Philips model TUV130WXPTDriver (Spain).

The average light intensity (31.1 W/m^2) that characterized the UV-C system was measured to ensure reproducibility of the process using a radiometer at 254 nm (Vilber Lourmat VLX, France). UV-C light dose was calculated as follows:

$$\text{UV-C light dose}\left(\text{J/m}^2\right) = \text{Average light Intensity}\left(\text{W/m}^2\right) \times \text{Exposure time (s)}.$$

A sensor was placed at the same height as the grape bunch in the middle of the lamp system. The UV-C system needed a lamp preheating of 10 min to stabilize the flow velocity.

2.4. Selective stilbene extraction method

The extraction method used was described in Guerrero et al. (2010a). Briefly: 5 ml of diethyl ether was added to 3 g of defrosted grape skin. Samples were ground using Ultraturrax T-25 equipment (Janke & Kunkel, Ika-Labortechnick, Deutschland, Germany) and stirred at 1200 rpm for 20 min. Once solutions were homogenous, they were centrifuged in a Digicen 20-R centrifuge (Orto Alresa, Spain) at 4053 *g* for 5 min, and 2 ml of the ether phase was decanted. The extraction was repeated but this second time a quantity of 4 ml was decanted. Finally, 6 ml of the ether phases (2 + 4 ml) was first dried with a vacuum centrifuge concentrator at room temperature. Dry samples were rediluted in 2 ml methanol, HPLC grade, and filtered through a 0.22 μm filter (PVDF Teknokroma, Barcelona, Spain). Samples were maintained in darkness and at low temperature (in foil film immersed in an ice bath) during the whole extraction process. Data are expressed as mg/kg of fresh weight (f.w.) skin.

2.5. Identification and quantification of stilbenes

Stilbenes were quantified as described by Guerrero et al. (2010a). Samples (20 μl) were analysed by a Waters HPLC system with a model 1525 pump and a Waters 996 Photodiode Array Detector. Separation was performed on a Mediterranea Sea18 column (Teknokroma) (RP-18, 25 × 0.46 cm; 5 μm particle size) and a guard column of the same material, at 30 °C. The mobile phases consisted of a water:methanol:acetic acid mixture, solvent A (88:10:2), and solvent B (8:90:2) at a flow rate of 1 ml/min. Stilbenes (*trans*-resveratrol, and ε-viniferin) were quantified at 306 nm as *trans*-resveratrol.

2.6. Statistical analysis

All samples were analysed in triplicate. Cluster analysis in harvest 2011 was conducted on average concentrations of *trans*-resveratrol at different days (before UV-C treatment, on harvest and at days 1, 2, 3 and 4 after harvest) to compare trends after treatment at different output powers. The Ward linkage method and the quadratic Euclidean distances were chosen as main parameters for the cluster analysis.

3. Experimental

UV-C light treatments were applied on plant shoot. Every shoot was randomly chosen for each experiment. A total of 60 shoots were available to be organized in experiments and control ones. Table 1 summarized experiment conditions explained below and experiment abbreviations used through this work.

3.1. Harvest 2010. Preharvest application day

Radiation conditions were fixed to determine the optimum application treatment day. 1 min treatment at maximum power (1040 W, 8 lamps on) was applied on shoots 7, 5, 3 and 1 days previous to harvest. Grapes were picked before and every 24 h after treatment until harvest to analyse stilbene content.