



## Research Paper

# Investigation of some physiological and chemical changes in shoots and leaves caused by UV-C radiation as an abiotic stress source in grapevine cuttings

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## ABSTRACT

In this study, UV-C was applied to the cuttings of Cabernet Sauvignon and Cabernet Franc cv.'s at three different durations to simulate abiotic stress, with subsequent monitoring of resultant changes upon the formation of at least six leaves. In both grape varieties, total phenolic compounds and antioxidant capacity in leaves reached the highest levels after 60 min of UV-C treatment. *Trans*-resveratrol content reached its highest level with an increase of 157 %. The activity of superoxide dismutase enzyme reached its highest level with an increase of 630 %, especially in Cabernet Franc. Total chlorophyll, chlorophyll a, chlorophyll b contents reached their highest levels after 60 min of UV-C treatment. UV-C treatments did not affect shoot number. However, parameters such as fresh and dry shoot weight, shoot length, fresh and dry root weight reached their highest levels after 45 min of UV-C stress. In correlation analysis, the highest correlation was found between *trans*-resveratrol and chlorophyll b. In principal component analysis, 6 min of UV-C stress had the highest loading on component 1 and 45 min of UV-C stress had the highest loading on component 2. These results suggest that UV-C treatment may be a potential method to improve grape quality and plant health by inducing important physiological responses in grapevines. This is the first study to investigate the effects of UV-C treatment on grapevine cuttings.

## 1. Introduction

Ultraviolet radiation (UVR) occupies at least 100 to 400 nanometers (nm) of the electromagnetic spectrum and the International Commission on Illumination (CIE) has divided the UV spectrum into three bands: UV-A from 315 to 400 nm, UV-B from 280 to 315 nm and UV-C from 100 to 280 nm (CIE, 1987, 1999a, 1999b; International Commission on Non-Ionizing Radiation Protection, 2004). The use of ultraviolet light is an effective way to induce secondary metabolites in many plants and the phenolic compound group is one of the important secondary compounds in plants (Mishra et al., 2020). UV light creates an abiotic stress in plant tissues and affects plant phenolic metabolites in different ways. It has been reported that UVB irradiation is associated with an increase in activity responsible for flavonoid biosynthesis and that these compounds can act as UV screens preventing UV-induced damage to the genetic material of plant cells (Cantos et al., 2000). Similarly, Bonomelli et al. (2004) reported that UV-C irradiation is a practical and reproducible method to trigger grapevine defense responses and may be

useful in determining the defense potential of grapevine cultivars. Significant differences in stilbene inducibility among *Vitis* genotypes in response to UV-C pulse have also been reported in previous studies (Duan et al., 2016). One of the most studied stilbenes among phenolic compounds is *trans*-resveratrol, which delays oxidative stress-induced apoptosis in various cell types due to its free radical scavenging and antioxidant properties (de la Lastra and Villegas, 2005). UV-C-induced increases in resveratrol were detected, which may also be related to the transcriptional regulation of genes involved in the production and signaling of secondary metabolites (Yin et al., 2016). The elicitor effects of UV-C on plant secondary metabolism have been attributed to the stimulation of reactive oxygen species (ROS) production by this radiation, thus requiring an enzymatic antioxidant mechanism to balance the excess ROS produced to prevent cell damage and even cell death (Urban et al., 2016). Plant cells develop an effective antioxidant defense system to cope with increased ROS. This system includes both enzymatic and non-enzymatic mechanisms. The enzymatic system is represented by enzymes such as superoxide dismutase (SOD), ascorbate peroxidase

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(APX), catalase (CAT) (Berli et al., 2010). Up-regulation of these enzymes in response to ROS production by abiotic stress factors has been observed (Gill and Tuteja, 2010; Maurer et al., 2017). In addition to stilbene content, UV radiation has also been reported to cause changes in chlorophyll concentrations in grapes (Pfundel, 2003; Sheng et al., 2018). As an effective method to improve plant growth and promote nutrient and bioactive compound accumulation, light radiation has been applied in crop production (Jones, 2018; Ma et al., 2020). Numerous studies have been conducted on the effect of ultraviolet radiation during cultivation (Caldwell, 1971; Tevini et al., 1981; Zuk-Golaszewska et al., 2003; Urban et al., 2016; Verdaguer et al., 2017; Vanhaelewyn et al., 2020; Tevini 2023), in postharvest (Liu et al., 2012; Abdipour et al., 2020; Yao et al., 2023), in plant tissue culture applications (Keskin and Kunter, 2010; Yıldız et al., 2023). In our previous studies, we have examined the effect of UV-C during grape fermentation (Tahmaz and Söylemezoglu, 2017) and in fermented wines (Tahmaz and Yüksel Küskü, 2022), however, to the best of our knowledge, there is no literature information on the changes caused by UV-C treatment of vine cuttings in young shoots and leaves. Therefore, in this study, grapevine cuttings of two different cultivars were rooted by applying UV-C for different times and then the following research questions were investigated in shoots and leaves: UV-C application times (1) whether it increases total phenolic compounds, antioxidant capacity and antioxidant enzyme levels of leaves, (2) increases *trans*-resveratrol levels, (3) changes chlorophyll and carotenoid levels, (4) affects rooting rate and some physical properties of shoots and leaves.

## 2. Materials and methods

### 2.1. Preparing of cuttings and UV-C treatments

One year old cuttings from Cabernet Franc clone 181 grafted on 41 B rootstock and Cabernet Sauvignon clone 169 (*Vitis vinifera* L.) grafted on 110 R rootstock, planted in 2003, were used as material in the study at the time of crop pruning in 2023 (March). Cuttings were obtained from a 2.1 × 1.0 m row spacing, cordon-trained vineyard of Chateau Kalpak (40°39'16.76"N, 27°03'18.74"E) in Şarköy, Tekirdağ, Turkey. Fifteen cuttings with 6 buds from each variety were delivered to Ankara University, Faculty of Agriculture, Department of Horticulture on the day of pruning and kept in cold storage at +4 ± 1 °C and 85 % humidity until UV-C treatment.

Grapevine cuttings were prepared on 04.04.2023 with one bud and exposed to UV-C irradiation in white polyethylene boxes at a height of 30 cm for 3 different times: 30 min, 45 min, 60 min. The UV-C lights consisted of a total of 4 lamps (Philips TUV PL-L) with a power of 36 W each and the UV irradiation of 1 lamp was 140 µW cm<sup>-2</sup> (Tahmaz and Yüksel Küskü, 2022). As soon as the treatment was completed, single-bud cuttings were planted in rooting pots containing peat, perlite, cocopeat, sand (1:1:1:1) and placed in climate-controlled cabinets at 24 °C for 12/12 h in light/dark and relative humidity of 70 %. Control grapevine cuttings were planted without UV-C treatment. The cuttings were removed from climate-controlled cabinets on 03.07.2023 (when shoots with a minimum of 6 leaves were formed from the cuttings) and analyzed on the same day. Each treatment contained 30 single-bud grapevine cuttings.

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$$\text{Rate of inhibition(\%)} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}}$$


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### 2.2. Extraction of leaf samples

To investigate the potential effects of UV-C irradiation on grapevine

cuttings, varying exposure durations of 30, 45, and 60 min were employed to assess alterations in total phenolic compounds, antioxidant capacity, and *trans*-resveratrol content in the leaves. Following separation from the shoots, the leaves were promptly frozen in liquid nitrogen and subsequently ground into a fine powder using a mortar and pestle. Extraction of phenolic compounds from the leaf samples was conducted utilizing a modified protocol derived from Duan et al. (2016). 600 mg fresh weight of powdered leaf tissue was taken into 50 mL centrifuge tubes, 5 mL of 100 % methanol was added and crushed in a homogenizer (Ultra-Turrax T25-Germany) for 5 min to maximize uniform sampling and ensure complete extraction. The samples in the tubes were then placed in an ultrasonic bath (Jeiotech US-Korea) at 30 °C for 10 min, followed by centrifugation at 10 000 rpm and 4 °C for 10 min (Sigma 3K30-Germany). At the end of centrifugation, the supernatant was removed and filtered using PVDF filters (Millipores-USA) with a pore size of 0.45 µm and stored at 4 °C for further analysis. Total phenolic compound and antioxidant capacity analyses by UV-VIS spectrophotometer were performed on the day of extraction, and *trans*-resveratrol analyses by LC-MS/MS were performed on the week of extraction.

### 2.3. Determination of total phenolic compounds

Total phenolic content of leaf extracts was determined according to Singleton and Rossi (1965). The absorbance values at 765 nm were measured by Shimadzu UV 1208 model UV VIS spectrophotometer (Japan) and the results were expressed in mg kg<sup>-1</sup> with the aid of the graph obtained by measuring the solutions prepared from Gallic Acid standards at concentrations of 50–4000 ppm (R<sup>2</sup>=0.998).

### 2.4. Determination of antioxidant capacity

Antioxidant capacity levels of the leaves were determined by 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods.

#### 2.4.1. ABTS

ABTS [2,2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)] method was performed according to Re et al. (1999) and the results were expressed as trolox equivalents (µmol trolox/g). For this purpose, 7 mM ABTS solution containing 2.45 mM potassium persulfate was prepared and the solution was kept at room temperature for 12–16 h to allow the formation of ABTS radical. For the dilution of ABTS and sample extracts, 8.77 g NaCl was added to 0.1 M phosphate buffer and the final volume was made up to 1 L with distilled water. Absorbance of the samples was recorded at 734 nm in disposable micro cuvettes. Before the analysis, 1 mL of ABTS radical solution was taken and diluted with phosphate buffer saline so that the absorbance at 734 nm was 0.700±0.02. The initial absorbance value was determined by taking 1 mL of the diluted solution into the cuvette and reading against PBS. Then, 10 µL of the extract was added to 990 µL of radical solution added to the cuvette with a final volume of 1 mL, and the absorbance value was recorded after 6 min. Percent inhibition rate was calculated according to the formula below.

The same procedure was then repeated for 20 and 30 µL sample volumes. The percentage inhibition values were plotted against the sample volumes (10, 20 and 30 µL) and linear regression analysis was

performed on the data obtained to obtain the curve of the sample and the equation describing this curve. The same procedure was performed for the trolox standard (R-(+)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 98 %-Aldrich 391,913) and the standard curve and the equation describing the curve were calculated. Shimadzu UV 1208 model UV VIS spectrophotometer (Japan) was used for the analysis. Results are expressed as  $\mu\text{mol Trolox g}^{-1}$ .

#### 2.4.2. DPPH

Stable radical DPPH solution was used in the measurements. The free radical scavenging effects of the extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95 %) were measured using the method modified by Lafka et al. (2007). To 0.1 mL of the sample solution prepared at different concentrations using methanol, 3.9 mL of DPPH solution prepared in methanol (25 mg/L) was added and mixed in a vortex for 30 s and kept for 30 min at room temperature and in the dark. After incubation in the dark, the absorbance of the samples was measured against methanol at 515 nm using a UV spectrophotometer. The free radical scavenging effects of the samples were calculated using the following formula.

$$\% \text{inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

$A_{\text{control}}$ : Absorbance value of 0.1 ml methanol + 3.9 ml DPPH (2,2-diphenyl-1-picrylhydrazyl) solution read against methanol

$A_{\text{sample}}$ : Absorbance value of the samples read against methanol after 30 min

The antioxidant activity was expressed as the  $\text{IC}_{50}$  value ( $\mu\text{g mL}^{-1}$ ) (concentration of the extract that inhibits 50 % of the production of DPPH).

#### 2.5. Trans-resveratrol assay

Trans-resveratrol analysis was performed at METU Central Laboratory, Molecular Biology, Biotechnology Research and Development Center, Mass Spectroscopy Laboratory, Ankara, Turkey, with Agilent 6460 LC-MS/MS. The equipment and operating conditions of the device are given in Table 1. Trans-resveratrol levels in leaf samples as a result of UV-C treatment are expressed in ppm.

**Table 1**  
LC-MS/MS equipments and conditions.

Equipment	Agilent 6460 LCMSMS
Ionisation source	ESI+Agilent Jet Stream
Pump	Agilent BinPump-SL (G1312B9)
Autosampler	Agilent h-ALS-SL+ (G1367D)
Column	Agilent G1316B 1200 Series Thermost. Col. Compart SL
Compartment	
Microdagger	Agilent T G1379B 1200 Series Micro Degasser
Software	Agilent T G3793AA Mass Hunter Optimizer software
Nitrogen Generator	Nitrogen generator UHPLCMS 30
Scan Mode	MRM
Gas Temperature	350 °C
Gas Flow	10 mL/min
Nebulizer	45 psi
Sheath Gas Temp.	350 °C
Sheath Gas Flow	10 mL/min
Capillary	4000 V
Nozzle Voltage	500 V
Liquid chromatography	
Equipment	Agilent 1200 HPLC Series
COLUMN	Zorbax SB-C18 (2,1 × 50 mm x 1,8 $\mu$ )
Mobile phase	Solvent A: 0,05% Formic Acid + 5 mM Ammonium Formate Solvent B: Methanol (MS grade, MERCK)
Column Temp.	40 °C
Flow	0,5 ml/min
Run Time	13 dak
Flow Mode	Gradient
Injection Volume	2 $\mu\text{L}$
Standart Curve Range	0.0001 - 1 ppm

#### 2.6. Antioxidant enzyme assays (CAT, SOD, APX)

One gram of fresh leaf tissue was homogenized ((Ultra-Turrax T25-Germany) with 5 mL of extraction solution containing 100 mM Naphosphate buffer, 1 mM EDTA- $\text{Na}_2$ , and 0.5 mM ascorbic acid adjusted to pH 7.6. The homogenized samples were subsequently centrifuged at 14,000 g for 30 min. The resulting supernatant served as a crude enzyme extract for the analysis of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) activities (Güneş et al., 2006). Catalase (CAT) activity was quantified by measuring the decrease in absorbance at 240 nm for one minute following the decomposition of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Cakmak et al., 1993). The reaction mixture (3 mL) consisted of 50 mM phosphate buffer (pH 7.0), 15 mM  $\text{H}_2\text{O}_2$ , and 50  $\mu\text{L}$  crude enzyme extract at 25 °C. The activity was calculated using the extinction coefficient for  $\text{H}_2\text{O}_2$  ( $40 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The superoxide dismutase (SOD) activity was quantified by the nitroblue tetrazolium (NBT) method, as previously described by Gong et al. (2005). The reaction mixture (3 mL) consisted of 50 mM potassium phosphate buffer, pH 7.3, 13 mM methionine, 75  $\mu\text{M}$  NBT, 0.1 mM EDTA, 4  $\mu\text{M}$  riboflavin, and enzyme extract (0.2 mL). Finally, riboflavin was added, and the glass test tubes were shaken and placed under fluorescent lamps ( $60 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). The reaction was allowed to stand for 5 min and then stopped by turning off the light. Absorbance was measured at 560 nm. Blanks and controls were run in the same way, but without illumination and enzyme extract, respectively. One unit of SOD was defined as the amount of enzyme that inhibited NBT reduction by 50 % under assay conditions. Ascorbate peroxidase (APX) activity was quantified by monitoring the decline in ascorbate concentration in a 2 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA- $\text{Na}_2$ , 0.5 mM ascorbic acid, 0.1 mM  $\text{H}_2\text{O}_2$ , and 50  $\mu\text{L}$  of crude enzyme extract. The absorbance change at 290 nm was recorded for 1 min, as described by Nakano and Asada (1981). The activity was calculated using the extinction coefficient for ascorbate ( $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ). All enzyme activities were made at 20° Shimadzu UV 1208 model UV VIS spectrophotometer (Japan). SOD activities were expressed as units per gram fresh weight of tissue ( $\text{U g}^{-1}$ ), CAT and APX activities were expressed as  $\text{mmol g}^{-1} \text{ min}^{-1}$ .

#### 2.7. Photosynthetic pigment assay

Chlorophyll and carotenoid measurements were performed according to Salachna et al. (2015). Briefly, 0.5 g of fresh leaf tissue was ground in a mortar with 15 ml of 80 % acetone to obtain a homogeneous extract, centrifuged at 10,000 rpm for 10 min, and the supernatant was separated. The absorbances of total carotenoids, total chlorophyll, chlorophyll a and chlorophyll b were recorded at 441, 646, 652 and 663 nm and calculations were made according to the following formulas. As a control, 80 % acetone was used and measurements were performed with a "Shimadzu" brand "UV-1280" model spectrophotometer (Japan).

$$\text{Chlorophyll a} (\text{mg g}^{-1}) = (12.21 \times A_{663} - 2.81 \times A_{646}) \times (V / 1000 \times m)$$

$$\text{Chlorophyll b} (\text{mg g}^{-1}) = (20.13 \times A_{646} - 5.03 \times A_{663}) \times (V / 1000 \times m)$$

$$\text{Total chlorophyll} (\text{mg g}^{-1}) = (27.8 \times A_{652}) \times (V / 1000 \times m)$$

$$\text{Total carotenoids} (\text{mg g}^{-1}) = [(1000 \times A_{441}) - 3.27 \times (12.21 \times A_{663} - 2.81 \times A_{646}) - 104 \times (20.13 \times A_{646} - 5.03 \times A_{663})] \times [V / 1000 \times (m \times 229)]$$

A is the absorbance at a specific wavelength, V is the volume of a volumetric flask in mL, and m is the weight of the sample in g.

## 2.8. Relative water content (RWC%)

After the leaf samples of the treatments were prepared in 1 cm<sup>2</sup> size, their fresh weights (FW) were determined. The turgor weights (TW) of the leaves were determined by keeping them in distilled water in a petri dish at 4 °C for 24 h, then drying them in an oven at 80 °C for 24 h and determining the dry weights (DW) of the samples. The relative water content (RWC%) of the leaves was calculated according to the following formula (Kırnak et al., 2001).

$$\text{RWC}(\%) = \frac{[\text{FW} - \text{DW}]}{(\text{TW} - \text{DW})} \times 100$$

## 2.9. Physical analysis of shoots and roots

In order to observe the morphological changes caused by UV-C treatments on shoot and root structure, each plant was uprooted from the rooting pots. The number of shoots was determined by counting the number of shoots and rooting rates were determined by counting the number of plants that rooted in each group (%). Shoot fresh weight (g), shoot dry weight (g), root fresh weight (g) and root dry weight (g) were determined using a three-stage analytical balance and shoot length (cm) was determined using a scale.

## 2.10. Data analysis

The experiment was carried out according to the randomized block design with two cultivars, three replicates, four UV-C treatments (including control), taking data from 30 cuttings in each treatment. Data were analyzed by analysis of variance (ANOVA) in SPSS version 14.0 statistical program and statistical analysis was performed by comparing the effects of treatments with Duncan's multiple comparison test. Correlation coefficients and principal component analysis were performed using JMP 13.2.0 statistical software.

## 3. Results

### 3.1. Effect of ultraviolet treatment of pre-planting cuttings on total phenolic compounds and antioxidant capacity of leaves

In the study, UV-C was applied for 30, 45, and 60 min to single-bud cuttings of Cabernet Sauvignon (CS) and Cabernet Franc (CF) varieties. Subsequently, the total phenolic compound (TPC) and antioxidant capacity (TEAC and IC<sub>50</sub>) levels of the leaves of the treatments were examined approximately 3 months later and are shown in Fig. 1 and Fig. 2. The 0 min measurement (0 min UV-C), serving as the control without UV-C treatment, was considered. The TPC, initially measured at 4264 mg GAE kg<sup>-1</sup> in 0 min UV-C CF, increased by 4.2 % with 30 min UV-C, 11 % with 45 min UV-C and 18 % with 60 min UV-C, reaching 5033 mg GAE kg<sup>-1</sup> ( $p \leq 0.05$ ). In accordance with the results of the UV-C application time, the CS variety exhibited incremental increases of 3.1 %, 7.6 %, and 12.1 % following 30, 45, and 60 min of UV-C exposure, respectively, ultimately reaching 6116 mg GAE kg<sup>-1</sup>. A significant observation across both cultivars was the consistent elevation in TPC content corresponding to the duration of UV-C exposure. The antioxidant capacities of leaf samples were investigated using two different spectrophotometric methods: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (Trolox equivalent antioxidant capacity-TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. According to the results shown in Fig. 2, TEAC, which was 34.86  $\mu\text{mol trolox g}^{-1}$  in the control group of CF, increased by 55 % with 30 min UV-C and reached 54.06  $\mu\text{mol trolox g}^{-1}$ , increased by 111 % with 45 min UV-C to 73.4  $\mu\text{mol trolox g}^{-1}$  and reached 82.06  $\mu\text{mol trolox g}^{-1}$  with an impressive increase of 135 % with the maximum UV-C time of 60 min ( $p \leq 0.05$ ). CS leaf samples followed the same trend as CF with increases of 16 %, 74 % and 129 %, respectively ( $p \leq 0.05$ ). It was concluded that the CF cultivar showed the highest rate of increase. The antioxidant activity results of the leaves by DPPH method were expressed using the term IC<sub>50</sub> (Fig. 2). The lower the IC<sub>50</sub>, the higher the antioxidant capacity. The highest IC<sub>50</sub> value in CF leaves was measured in UV-C samples applied for 45 and 60 min, and the second highest value was 30 min UV-C ( $p \leq 0.05$ ). In CF samples, 45 min and 60 min applications did not make a statistical

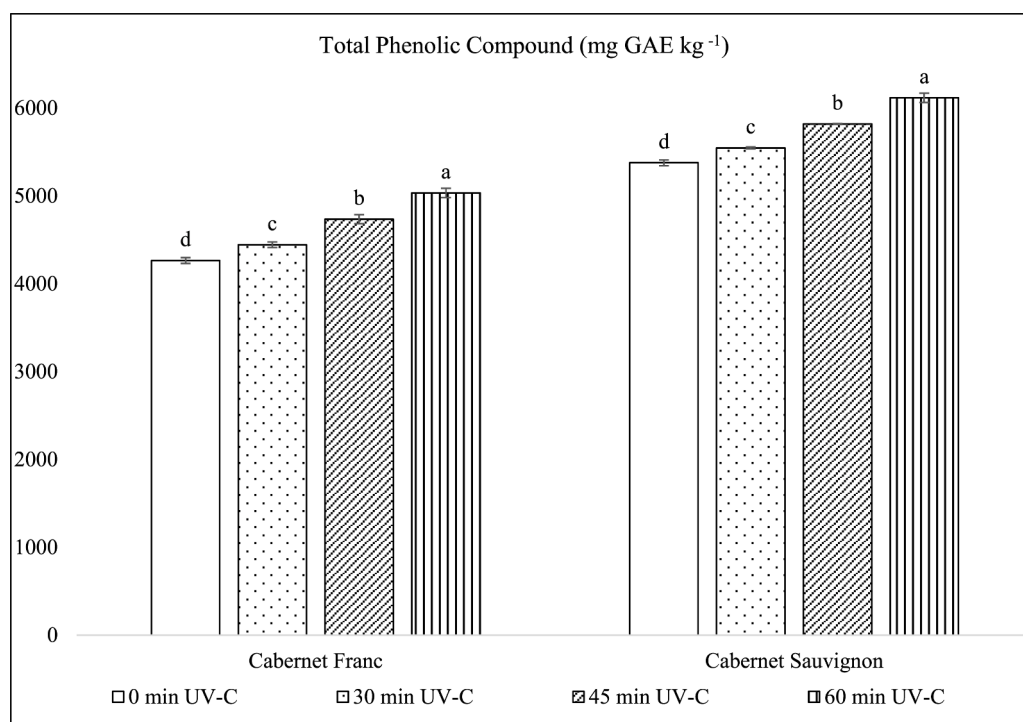


Fig. 1. Changes in total phenolic compound levels of leaves caused by UV-C irradiation applied to cuttings for 0, 30, 45 and 60 min before planting.

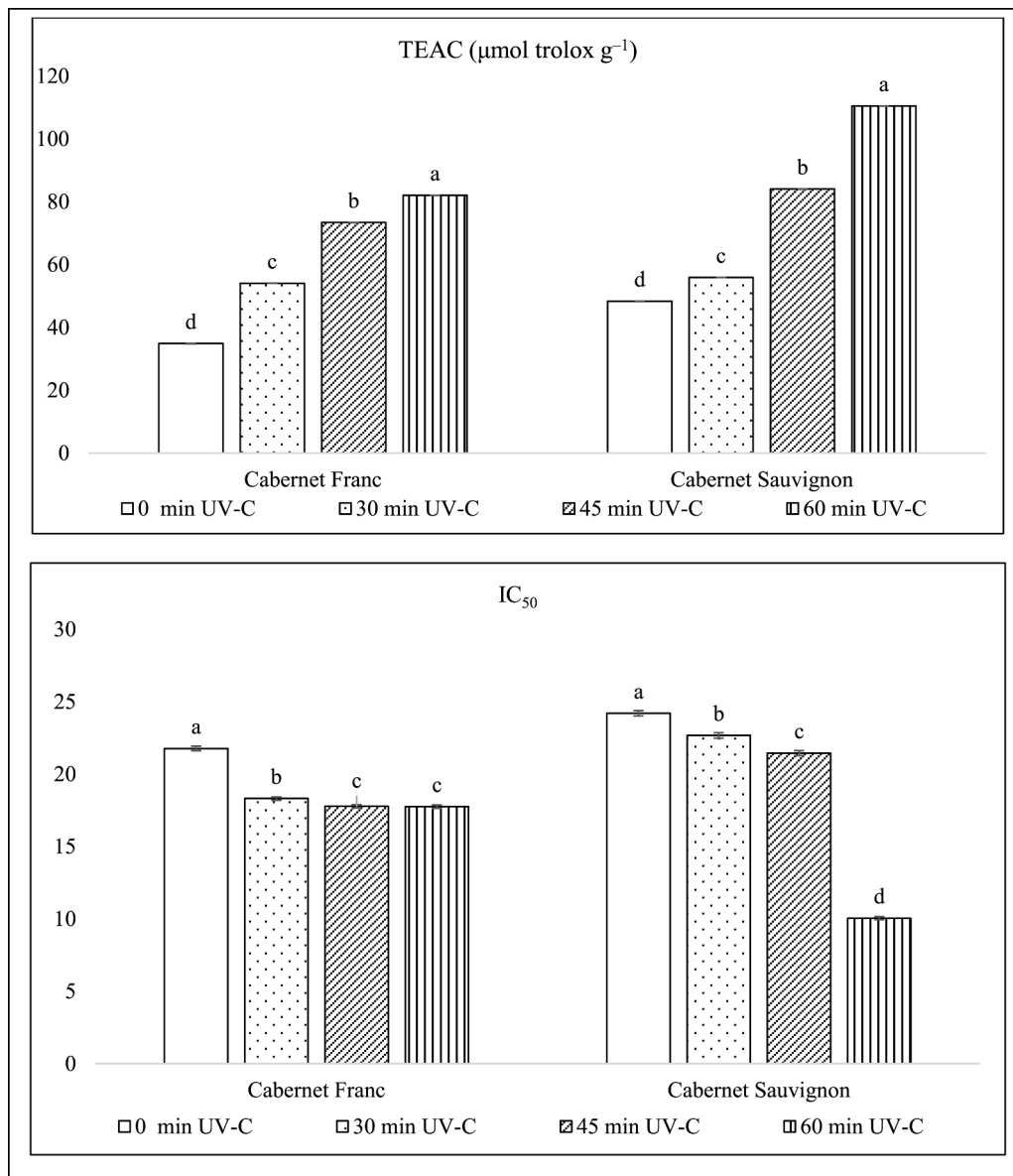


Fig. 2. Changes in antioxidant capacity levels of leaves caused by UV-C irradiation applied to cuttings for 0, 30, 45 and 60 min before planting.

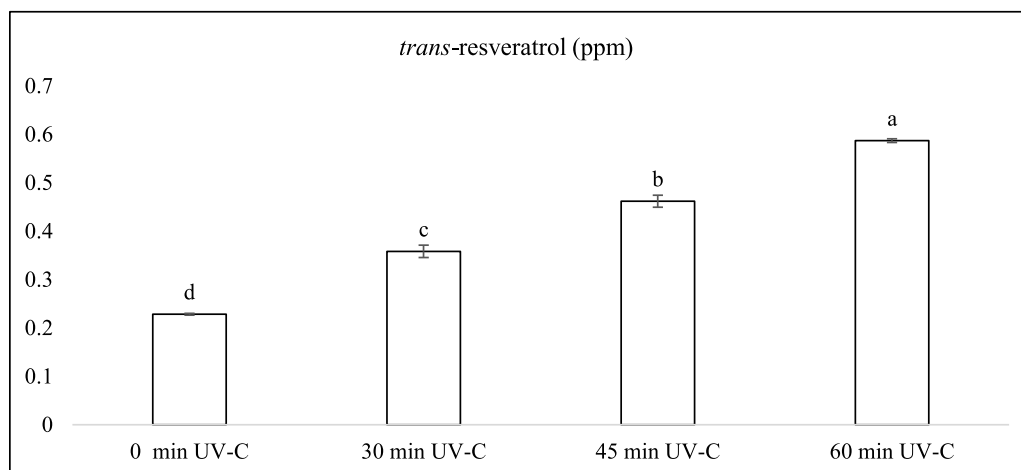


Fig. 3. Changes in *trans*-resveratrol levels of CF leaves caused by UV-C irradiation applied to cuttings for 0, 30, 45 and 60 min before planting.

difference. In CS variety, the highest antioxidant capacity level with 58 % increase was reached with 60 min UV-C ( $p \leq 0.05$ ).

Each value represents the mean  $\pm$  standard error of three replicates. Within the varieties, the means shown with different letters in each graph are significantly different ( $p \leq 0.05$ ). Vertical bars correspond to standard error.

Each value represents the mean  $\pm$  standard error of three replicates. Within the varieties, the means shown with different letters in each graph are significantly different ( $p \leq 0.05$ ). Vertical bars correspond to standard error.

### 3.2. Effect of ultraviolet treatment of pre-planting cuttings on trans-resveratrol levels in leaves of cabernet franc

The study was planned to determine the amount of *trans*-resveratrol in both cultivars, but was conducted only in the CF cultivar. For this reason, the differences in the amount of *trans*-resveratrol in the leaves of the CF variety caused by UV-C exposure at different times are shown in Fig. 3. In our study, the amount of *trans*-resveratrol in the leaves of the 0 min UV-C group, in which no UV-C was applied to the preplanting cuttings, was measured to be 0.2283 ppm. This value increased by 57 %

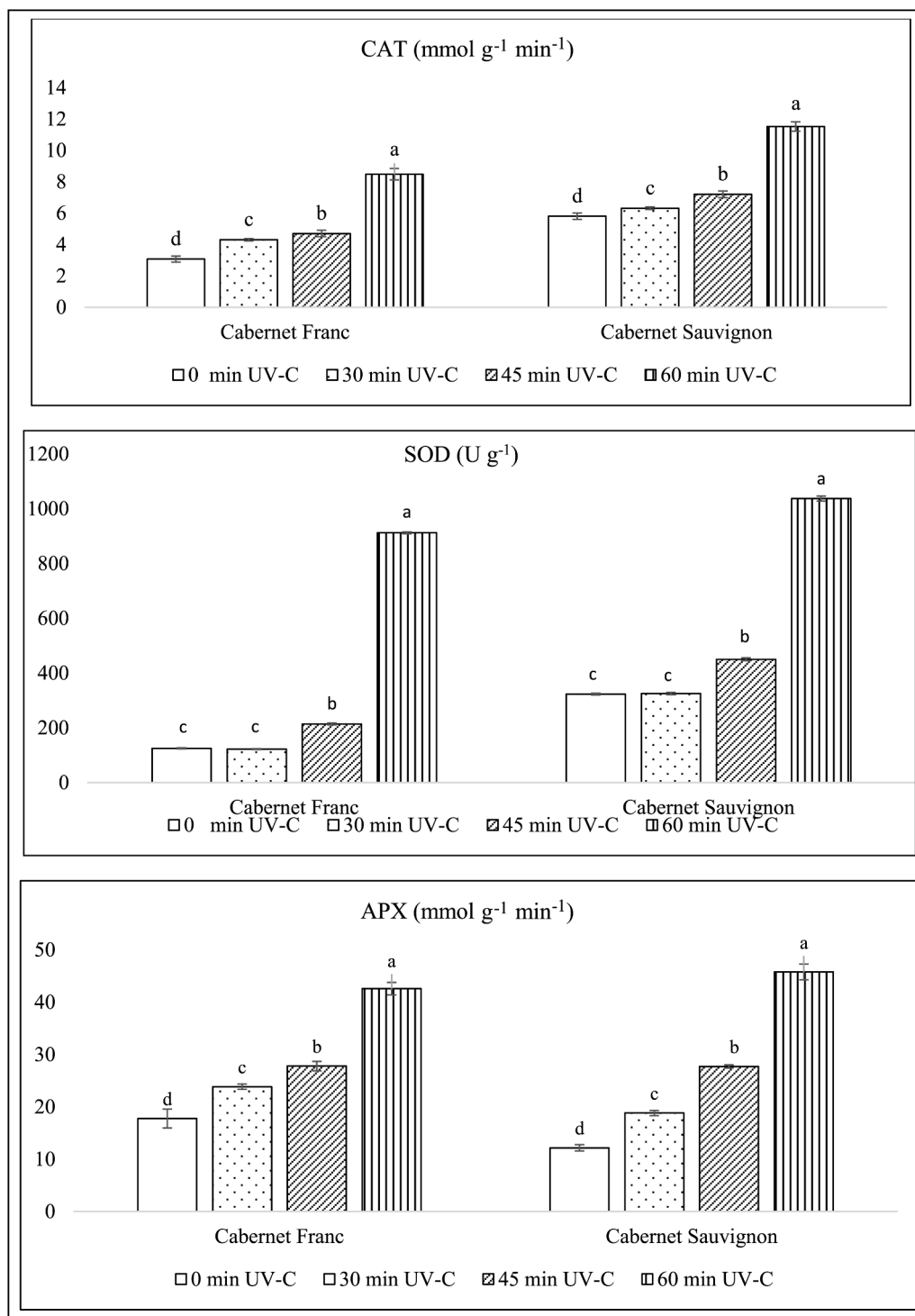


Fig. 4. Changes in antioxidant enzymes of leaves caused by UV-C irradiation applied to cuttings for 0, 30, 45 and 60 min before planting.

to 0.3583 ppm with 30 min UV-C and by 102 % to 0.4619 ppm with 45 min UV-C ( $p \leq 0.05$ ). One of the most significant results of the study was that *trans-resveratrol* levels in cuttings increased by 157 % to 0.5871 ppm with 60 min UV-C exposure ( $p \leq 0.05$ ).

Each value represents the mean  $\pm$  standard error of three replicates. Within the variety, the means shown with different letters in each graph are significantly different ( $p \leq 0.05$ ). Vertical bars correspond to standard error.

### 3.4. Effect of ultraviolet treatment on antioxidant enzyme levels of leaves in pre-planting cuttings

The antioxidant enzyme levels of the leaves are given in Fig. 4. It is noticed that as the UV-C stress duration of the samples increased, catalase (CAT) activities were also increased ( $p \leq 0.05$ ). The CAT activities of the leaves of CF and CS cultivars showed the highest increase of 176 % and 99 %, respectively, as a result of 60 min UV-C ( $p \leq 0.05$ ). CAT level increased to 4.3 mmol g<sup>-1</sup> min<sup>-1</sup> with 30 min application, 4.7 mmol g<sup>-1</sup> min<sup>-1</sup> with 45 min application and 8.5 mmol g<sup>-1</sup> min<sup>-1</sup> with 60 min application ( $p \leq 0.05$ ). Conversely, superoxide dismutase (SOD) activity values of both varieties did not show a significant increase in 0 min and 30 min UV applications compared to the control group ( $p \leq 0.05$ ). However, SOD level increased as the exposure time increased. Notably, 45 min UV-C significantly increased SOD activity by 71 % and 39 % in CF and CS, respectively. The highest SOD activity occurred as a result of 60 min UV-C as in CAT. However, there was a much higher increase in activity compared to CAT and an increase of 630 % was measured especially in CF ( $p \leq 0.05$ ). Similar to CAT measurements, ascorbate peroxidase (APX) values also increased parallel to UV-C application times. The value of 17.75 mmol g<sup>-1</sup> min<sup>-1</sup> in the CF control group increased by 34 % to 23.83 mmol g<sup>-1</sup> min<sup>-1</sup> in 30 min samples, 56 % to 27.77 mmol g<sup>-1</sup> min<sup>-1</sup> in 45 min samples and 140 % to 42.58 mmol g<sup>-1</sup> min<sup>-1</sup> in 60 min samples ( $p \leq 0.05$ ). A similar increase in APX activities was observed in CS samples. The most effective increase was 277 % in 60 min samples ( $p \leq 0.05$ ). Overall, antioxidant enzyme activities tended to increase parallel to the time except SOD 30 min treatment.

Each value represents the mean  $\pm$  standard error of three replicates. Within the varieties, the means shown with different letters in each graph are significantly different ( $p \leq 0.05$ ). Vertical bars correspond to standard error. CAT: Catalase, SOD: Superoxide dismutase, APX:

Ascorbate peroxidase, U: Unite.

### 3.5. Effect of ultraviolet treatment of pre-planting cuttings on photosynthetic pigment levels of leaves

Changes in photosynthetic pigment content of leaves induced by 30, 45 and 60 min UV-C stress applied to CF and CS cuttings are shown in Fig. 5. The total chlorophyll content of the control group of CF cultivar increased from 1.72 mg g<sup>-1</sup> at 0 min UV-C to 2.69 mg g<sup>-1</sup> by 33 % at 30 min UV-C, 57 % at 45 min UV-C and 56 % at 60 min UV-C ( $p \leq 0.05$ ). Similarly, in the CS, increases in the same trend were observed and the highest value was measured as 2.97 mg g<sup>-1</sup> after 60 min UV-C. Chlorophyll a and chlorophyll b contents also tended to increase in parallel with UV time ( $p \leq 0.05$ ). Chlorophyll a, which is the predominant chlorophyll in the leaves, increased by 22 %, 26 %, and 40 %, and chlorophyll b increased by 14 %, 21 %, and 40 %, respectively, as a result of 30, 45, and 60 min UV-C compared to the CF control group. In CS, this increase was 23 %, 39 %, 54 % for chlorophyll a and 17, 48, 70 % for chlorophyll b ( $p \leq 0.05$ ). Notably, the highest chlorophyll a was 2.03 mg g<sup>-1</sup> in CS and the highest chlorophyll b was 0.59 mg g<sup>-1</sup> in CF. Chlorophyll a was higher in CS and b was higher in CF. Conversely, the amount of carotenoids exhibited less variation in response to UV-C exposure compared to other pigments. Accordingly, an increase of 5 %, 17 %, 20 % was observed in CS with 30, 45, 60 min applications ( $p \leq 0.05$ ). In CF, the highest amount of caretonoid was 2.57 mg g<sup>-1</sup> with 30 min UV-C. This value was followed by 60 min and 45 min UV-C treatments, with increases of 29 % and 21 %, respectively.

Each value represents the mean  $\pm$  standard error of three replicates. Within the varieties, the means shown with different letters in each graph are significantly different ( $p \leq 0.05$ ). Vertical bars correspond to standard error.

### 3.6. Effect of ultraviolet treatment of pre-planting cuttings on some physical properties of shoots, leaves and roots

Some physical changes on shoots, leaves, and roots induced by UV-C application to cuttings at different times are detailed in Table 2. The number of shoots was consistent across both cultivars, indicating a single shoot formation per cutting. Fresh shoot weight (FSW) values of CF and CS cultivars reached their peak after 45 min of UV-C exposure,

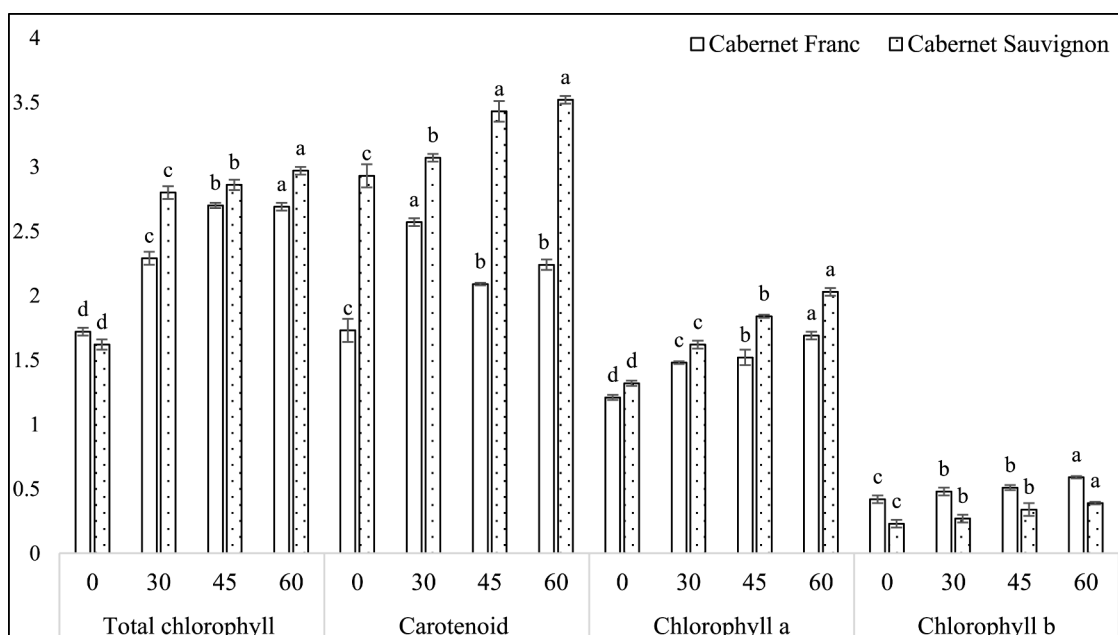


Fig. 5. Changes in photosynthetic pigments of leaves caused by UV-C irradiation applied to cuttings for 0, 30, 45 and 60 min before planting.

**Table 2**

Changes in some physical properties of shoots, leaves and roots by UV-C irradiation applied to cuttings for 0, 30, 45 and 60 min before planting.

		Cabernet Franc	Cabernet Sauvignon
Number of shoots	0	1 NS	1NS
	30	1 NS	1NS
	45	1 NS	1NS
	60	1 NS	1NS
Fresh shoot weight (g)	0	1.09±0.03c	0.99±0.12 c
	30	1.56±0.16 b	1.69±0.27 b
	45	2.35±0.07 a	2.41±0.08 a
	60	0.30±0.04 d	0.47± 0.06 d
Dry shoot weight (g)	0	0.19±0.07 b	0.28±0.04 b
	30	0.25±0.03 b	0.32±0.06 b
	45	0.57±0.03 a	0.61±0.04 a
	60	0.05±0.01 c	0.09±0.03 c
Shoot length (cm)	0	12.70±0.84 c	12.81±0.90 c
	30	15.90±0.32 b	17.87± 0.27 b
	45	20.00±0.58 a	22.40±0.60 a
	60	3.80±0.17 d	6.20± 0.21 d
Fresh root weight (g)	0	0.23± 0.03 b	1.14±0.06 b
	30	0.46±0.01 b	1.37±0.02 b
	45	1.27±0.01 a	2.18±0.04 a
	60	0.06±0.03 c	2.37±0.01 a
Dry root weight (g)	0	0.03±0.01 b	0.09±0.00 b
	30	0.07±0.01 b	0.05±0.01 c
	45	0.21±0.02 a	0.19±0.0 a
	60	0.01±0.00 b	0.17± 0.11 a
Rooting rate (%)	0	100±0 a	100 NS
	30	100±0 a	100 NS
	45	100±0 a	100 NS
	60	80±0 b	100 NS
RWC (%)	0	90.67 NS	89.46±3.51 b
	30	90.22 NS	88.01±2.63 b
	45	91.68 NS	90.47±2.86 b
	60	92.97 NS	94.76±0.51 a

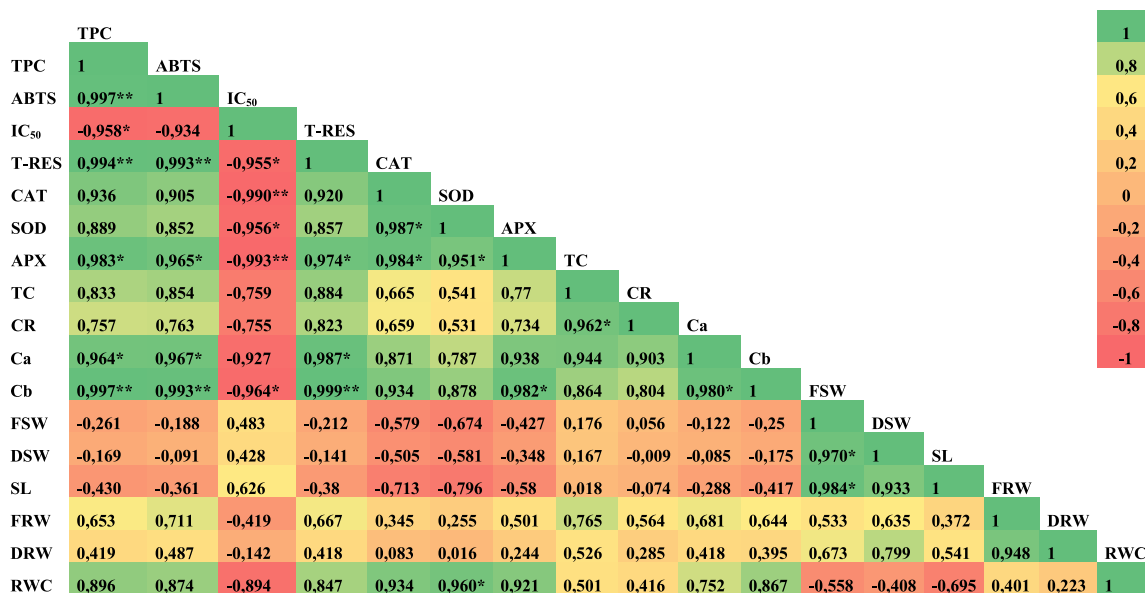
NS: Not significant. Different letter indicates significant differences for each parameter within coloumn ( $p \leq 0.05$ ). RWC: Relative water content.

exhibiting a notable increase of 116 % in CF and 143 % in CS ( $p \leq 0.05$ ). Subsequently, the second-highest increases were observed with 30 min of UV-C application, with increments of 43 % in CF and 71 % in CS ( $p \leq 0.05$ ). Conversely, fresh shoot weights (FSW) of both varieties displayed

a decrease following 60 min of UV-C exposure. In the control group, fresh shoot weights decreased significantly from 1.09 g and 0.99 g to 0.3 g and 0.47 g in CF and CS, respectively ( $p \leq 0.05$ ). Dry shoot weight (DSW) alterations mirrored those of fresh shoot weight changes. Notably, the highest dry shoot weight was recorded after 45 min of UV-C exposure (CF: 0.57 g, CS: 0.61 g). As expected, akin to fresh shoot weight, a 60-minute UV-C treatment led to a substantial decrease in dry shoot weight by 72 % in CF and 68 % in CS ( $p \leq 0.05$ ). Fresh root weight emerged as the most notably affected parameter among the physical parameters post-UV-C treatments. The most significant increase in root weight was observed in CF, with a remarkable 452 % increase induced by 45 min of UV-C exposure ( $p \leq 0.05$ ). In CF, 30 min showed no significant difference in fresh root weight compared to the control group, whereas a 74 % decrease was noted following 60 min of UV-C exposure. Conversely, in CS, fresh root weight values remained largely consistent with the control group at 30 min, but increased by 100 % at 45 and 60 min ( $p \leq 0.05$ ). Dry root weight (DRW) levels exhibited changes only with 45 min of UV-C exposure in CF, while in CS, it decreased by 44 % at 30 min of UV-C exposure and increased at 45 and 60 min with statistically similar significance ( $p \leq 0.05$ ). The longest shoot lengths (SL) were obtained at 45 min after UV-C exposure (CF: 20 cm, CS: 22.4 cm), while 60 min of exposure caused shoots to be even shorter than those in the control group ( $p \leq 0.05$ ). Relative Water Content (RWC%) levels reached the highest level of 64.76 % in CS. Conversely, the lowest RWC values were observed in the control group, with RWC rates increasing as the treatment duration lengthened ( $p \leq 0.05$ ). All rooting rates of the cuttings were 100 %, except for CF following 60 min of UV-C exposure. UV-C application to the cuttings did not induce significant changes in RWC rates compared to other parameters. In CF, RWC values ranged between 90.67 % and 92.97 %, while in CS, the highest RWC value of 94.76 % was observed after 60 min of UV-C exposure ( $p \leq 0.05$ )

3.7. Correlation matrix analysis

The interrelationships of the parameters analyzed in the study are presented in Fig. 6. The correlation coefficient (positive or negative) is represented by the virtual color as shown in the color key. The correlation coefficient becomes greenish as it approaches 1 and reddish as it



**Fig. 6.** Correlation matrix analysis between the data of the studied parameters. TPC: Total phenolic compound, T-RES: *Trans-resveratrol*, CAT: Catalase, SOD: Superoxide dismutase, APX: Ascorbic peroxidase, TC: Total chlorophyll, CR: Caretonoid, Ca: Chlorophyll a, Cb: Chlorophyll b, FSW: Fresh shoot weight, DSW: Dry shoot weight, SL: Shoot length, FRW: Fresh root weight, DRW: Dry shoot weight, RWC: Relative water content. The correlation values ranged from -1 (coloured in reddish) to 1 (coloured in greenish). Single asterisk “\*” indicates significant differences at the level of  $p \leq 0.05$ , and double asterisks “\*\*” indicate significant differences at the level of  $p \leq 0.01$ .

approaches  $-1$ . The parameters with the highest statistically significant positive correlation are (*trans*-resveratrol) T-RES and chlorophyll *b* (Cb) ( $R = 0.999, p \leq 0.01$ ), Cb is positively correlated with total phenolic compound (TPC) with an R value of 0.997 at the same significance level. At 0.05 significance level, the highest positive correlation is between SOD and CAT. The parameters with significant negative correlation with each other were chlorophyll *b* and  $IC_{50}$  ( $R = 0.964, p \leq 0.05$ ). The correlation levels of chlorophyll *b* with total phenolic compound, ABTS, *trans*-resveratrol, APX and chlorophyll *a* levels are interesting. The same remarkable result is also seen in the relationships between relative water content and SOD and chlorophyll *a* and total phenolic compound, ABTS, *trans*-resveratrol.

### 3.8. Principal component analysis (PCA)

Principal component analysis (PCA) was used to evaluate the interaction between UV-C stress treatments of different durations and the variables studied, as shown in Fig. 7. A data set of 4 treatments (0 min UV-C, 30 min UV-C, 45 min UV-C, 60 min UV-C) and 17 variables was analyzed using the covariance matrix. The results of the PCA revealed a three-component model that explained 99.98 % of the total variance. The first principal component explained 67.8 % of the total data variance, while the other components explained 27.1 % and 5.08 %, respectively. Individual loadings showed that 60 min UV-C had the highest loadings on Component 1, while 45 min UV-C had the highest loadings on Component 2, indicating a positive correlation with these components. Based on these loadings, it can be concluded that the 45 min UV-C variable is important in explaining the variation in the data along the second principal component, while the 60 min UV-C variable is important in explaining the variation in the data along the first principal component. The control treatment 0 min UV-C has negative loadings on both components and 30 min UV-C has a negative loading on component 1. The fact that the variables DRW, FRW, TC, CR, Ca,

ABTS, T-RES, Cb, TPC, APX, RWC, CAT and SOD have high positive loadings on Component 1 indicates that they have a strong relationship with the first principal component and are important in explaining the variation in the data along this dimension. The APX, RWC, CAT, and SOD variables have positive loadings on Component 1 and moderate negative loadings on Component 2, indicating that they are important in explaining the variation in the data along the first component. The variable  $IC_{50}$  has a high negative loading on Component 1 and a negative relationship along this component. The variables SL, FSW, and DSW have high positive loadings on Component 2, indicating a high correlation with the second component. The angles between the components and the commonalities between the components in the PCA plot provide information about the strength of the correlation between the original variables. Narrower angles indicate higher correlation, while wider angles indicate lower correlation, in other words, similar observations tend to be close together. Accordingly, Ca, ABTS, T-RES, Cb and TPC variables have a high positive correlation with each other. RWC and CAT variables also show similar characteristics. The highest negative correlation is between  $IC_{50}$  and SOD, where the angle between them is the largest.

## 4. Discussion

Vine and grape tissues are known to contain high levels of antioxidant compounds (Radulescu et al., 2020; Küpe et al., 2021; Baroi et al., 2022). In the context of sustainable viticulture, innovative approaches such as elicitor-induced stimulation of immunity and quality parameters have been intensively studied in recent years. Elicitors are substances that cause physiological changes in plants. Plants respond to these stressors by activating a series of mechanisms that affect plant metabolism and increase the synthesis of phytochemicals, similar to defense responses to pathogen infections or environmental stimuli (Baenas et al., 2014). UV-C treatment is also one of the abiotic elicitors and the subject

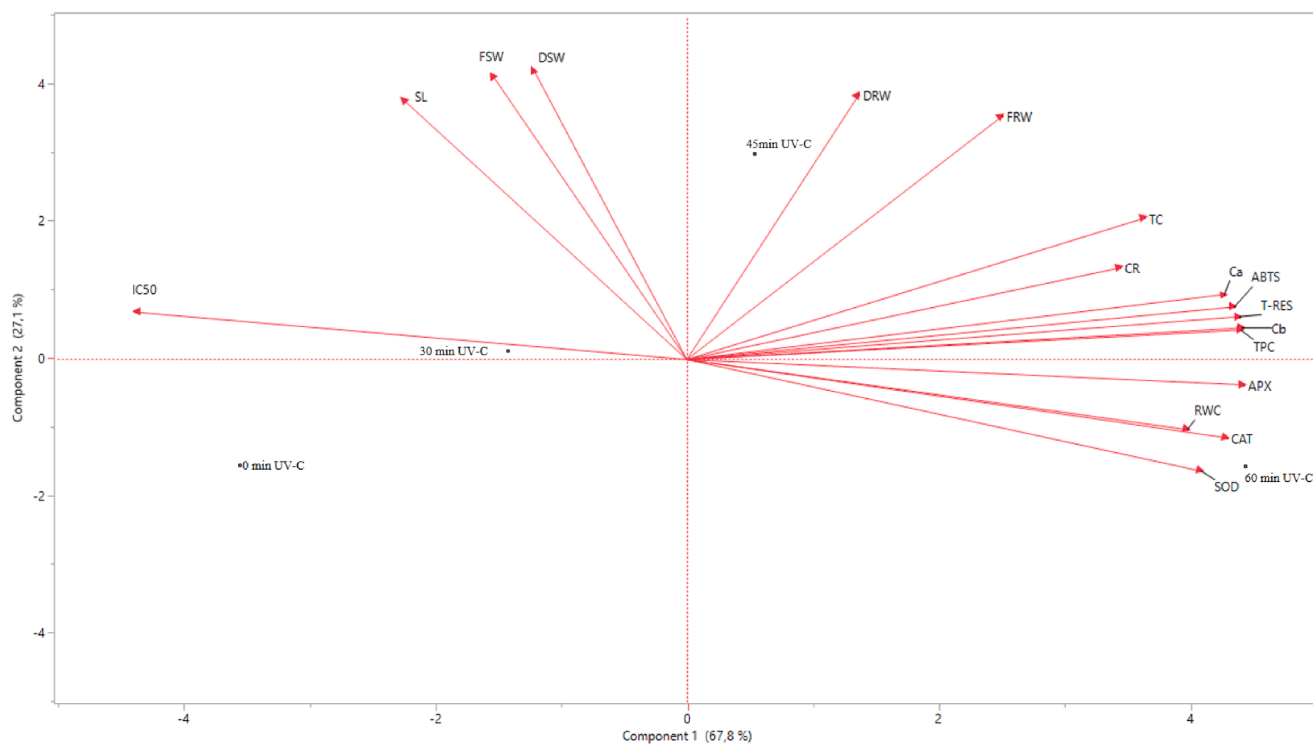


Fig. 7. The biplot of scores and loadings for the Principal Component Analysis (PCA) of all data shows the distribution of reaches according to the first two components. The size of the red arrows indicates the strength of the contribution of the 17 variables. TPC: Total phenolic compound, T-RES: *Trans*-resveratrol, CAT: Catalase, SOD: Superoxide dismutase, APX: Ascorbic peroxidase, TC: Total chlorophyll, CR: Caretonoid, Ca: Chlorophyll *a*, Cb: Chlorophyll *b*, FSW: Fresh shoot weight, DSW: Dry shoot weight, SL: Shoot length, FRW: Fresh root weight, DRW: Dry shoot weight, RWC: Relative water content.

of this study was to apply UV-C irradiation to grapevine cuttings for different durations to obtain maximum antioxidant enzymes and activity by observing some chemical and physical changes in the leaves of the shoots. Although many studies have focused on UV-B and UV-A rather than UV-C, some studies have pointed out that UV-C has many regulatory effects on plant morphology, physiology and biochemistry and that hormetic doses of UV-C light can stimulate secondary metabolism and thus the accumulation of secondary metabolites, especially phenolic compounds (Bonomelli et al., 2004; Xi et al., 2014; Del-Castillo-Alonso et al., 2015, 2015; Urban et al., 2018).

Elicitation of plant raw materials through pro-oxidant factors is based on the phenomenon of hormesis, according to Piechowiak (2024). This means that the stimulating effect of elicitors on the plant is only observed in the case of low doses or short-term effects of the agent. In the present study, the minimum UV-C duration was 30 min and the maximum UV-C duration was 60 min, and the main characteristic that distinguished our study from others was that UV was applied to pre-planting cuttings and not to green leaves. Of all UV radiation, only UV-C does not penetrate the earth's surface in significant amounts due to its high level of absorption by the ozone layer (Urban et al., 2016). This explains why UV-C light has been neglected in the last two decades while much research has been conducted on the biological effects and mechanisms of action of UV-B and UV-A radiation. In our research results, the highest TPC and AC contents of leaves after UV-C stress were obtained after 60 min UV-C, which means that the increase in these parameters was parallel to the UV-C duration (Fig. 1). It has been reported that one of the main damages caused by UV radiation is oxidative stress, which leads to excessive ROS production that damages DNA, proteins and lipids (Pathak et al., 2019), inducing an increase in antioxidant capacity in response to stress. Ranjbaran et al. (2021) reported that TPC contents in UV-C treated Thompson Seedless cultivar were measured significantly higher than the control during storage and phenolic elevation in plants under UV stress is a kind of defense response. Wu et al. (2016) reported that all tissues of UV-C treated mushrooms had higher DPPH scavenging activity than controls during storage, Shen et al. (2013) similarly reported that flavonoids and TPC content in Satsuma mandarins increased significantly with 1.5 and 3.0 kJ/m<sup>2</sup> treatments during 3 days of storage, Wang et al. (2009) reported that different UV-C doses caused different degrees of response in blueberry fruit and all UV-C doses increased TPC levels compared to the control group. It can be concluded that under the influence of UV, it can biosynthesize antioxidant compounds as endogenous defense mechanisms that produce radical oxygen species.

Stilbenes are a class of secondary metabolites that are crucial for grapevine basal immunity (Bavaresco et al., 2009) and play an important role in response to stresses (Jeandet et al., 2002). They are constitutively produced at low levels, but their levels increase significantly when plants are exposed to stressful conditions (Luo et al., 2019). UV-C radiation can modulate secondary metabolite biosynthesis and these increased amounts of metabolites act as scavengers for free radicals and protect cell damage from irradiation (Schreiner et al., 2014). UV-C has also been reported to induce the activity of various secondary metabolic enzymes and in our study, the highest levels of *trans*-resveratrol were obtained after prolonged UV-C (Fig. 3) (Mishra et al., 2020). Similar results to those in our study were demonstrated in Xi et al. (2015) with a 137 % increase in *trans*-resveratrol content at 48 h after UV-C irradiation of grapevine leaves. Kiselev et al. (2019) reported that after UV-C exposure to leafy cuttings of *Vitis Amurensis*, the total content of stilbenes increased 2.9-fold and *trans*-resveratrol increased 2.5-fold. Previous studies have emphasized that UV-C irradiation significantly stimulates resveratrol accumulation in leaves regardless of developmental stage (Wang et al., 2016) and is an excellent method for induction of secondary metabolites in many plants, in particular, low doses of UV-C for up to 30 min trigger key enzymes of their metabolic pathways, allowing plants to partially return to their normal physiological state, resulting in a significant increase in the secondary metabolite (Mishra

et al. 2020). The fact that *trans*-resveratrol accumulated more as a result of high UV-C can be explained by the fact that the stability of phenolic compounds present in plants can be increased and phenolic compounds accumulate and cause longer-lasting antioxidant activity. Similar results were reported by Shen et al. (2013). Douillet-Breuil et al. (1999) also confirmed the role of *trans*-resveratrol in the active defense mechanisms of grapevines when UV is used as a protectant in grapevine leaves. UV radiation can also be used for different purposes. For example, Keskin and Kunter (2008) used UV-C radiation to induce *trans*-resveratrol production in grapevine callus cultures and Pinto et al. (2022) used UV-C radiation to increase this compound in postharvest grapes.

Remarkably, less is known about UV-C light, as it seems to have the potential to be more effective than UV-B light. Recent studies have shown that UV-C light as an abiotic stress plays important roles in the ontogeny of plants, affecting postharvest physiology, secondary metabolism and plant defense, and can induce the expression of antioxidant enzymes and accumulation of compounds such as anthocyanins, flavonoids, phenolic acids (Liu et al., 2012; Dou et al., 2019; Moreira-Rodríguez et al., 2017). The results of the study showed similar findings in antioxidant enzyme activities as in TFB and AC results. The highest enzyme activity was observed as a result of UV-C for the maximum period of treatment (Fig. 4). SOD activity has been shown to be increased by a large number of ROS-producing stress factors and is also associated with increased tolerance to abiotic stress (Sharma et al., 2012). Since the coordinated functions of antioxidant enzymes such as SOD, POD and CAT help in the processing of ROS and the regeneration of redox ascorbate and glutathione metabolites, CAT activity is also associated with the scavenging of H<sub>2</sub>O<sub>2</sub> and its increased activity is associated with increased stress tolerance (Gill and Tuteja, 2010). Nantapong et al. 2019; Berwal et al. 2021). Antioxidant enzyme activities increased as the intensity of stress factors such as UV radiation increased. In studies applying post-harvest UV-C irradiation, it was emphasized that grape antioxidant enzymes and activities increased (Maurer et al., 2017), and in another study, low temperature stress increased SOD and CAT activities in wine grape seedlings (Wang et al., 2023). The increase in antioxidant enzymes is thought to be a response to the development of defense mechanisms against UV-C application as mentioned by Bonomelli et al. (2004). In studies where UV-C was applied to peanut sprouts (Zhu et al., 2021), strawberries (Li et al., 2019), it was reported that antioxidant enzymes increased compared to the control group.

Chlorophyll is a complex molecule consisting of four pyrrole rings, a magnesium atom and a long phytol chain and is crucial for photosynthesis, and since changes in chlorophyll content reflect photosynthetic capacity (Berg et al., 2002; Cutraro and Goldstein, 2005), to some extent they also reflect the ability of plants to resist stress (Guerfel et al. 2009). The photosynthetic process is highly sensitive to any change in the environment and chlorophyll is the photosynthetic pigment involved in the absorption, transmission, distribution and conversion of light (Zhang et al., 2014). Carotenoids are localized in the plastids of both photosynthetic and non-photosynthetic tissues (Sivritepe, 2001), act as light harvesting pigments (Xiao et al., 2017) and also have antioxidant effects (Young and Lowe, 2001) because they have the ability to scavenge reactive oxygen species (ROS) (Soares Netto, 2001). The high levels of carotenoid and antioxidant capacity both improved with increasing UV-C duration (Fig. 5). This result may be explained by the fact that UV-C light affects the release of signaling molecules in plants, triggering the genetic regulation of antioxidant enzymes and other defense mechanisms, thus increasing antioxidant activity. Our results show that chlorophyll levels increase with increasing UV-C exposure time (Fig. 5). In line with our results, Luo et al. (2019) and Doupis et al. (2020) reported that under UV stress, some grape genotypes developed the ability to increase chlorophyll metabolism and carotenoid accumulation as a defense response. In this way, it can be concluded that by increasing the photosynthesis-related processes of the plant, photosynthetic performance can also be regulated and plant growth can be stimulated.

Although it seems to have the potential to be effective in a shorter period of time than UV-B light, much less is known about UV-C light (Urban et al. 2016). Data on the effects of UV-A and UV-B on plant growth in different species are readily available, but studies on the effects of UV-C are scarce (Yuan et al., 1998; Zhang et al., 2014; Qian et al., 2020; Tevini, 2023). As a result of the study, RWC% levels were not affected by the duration of stress in CF, while the highest RWC in CS was the result of 60 min UV-C (Table 2). Even though a previous study reported that UV-C treatments did not change the physical properties of grapevine (Gadoury et al., 2022), the changes in physical parameters related to shoots and roots in this study are interesting: FSW, DSW, SL, FRW, DRW reached their highest level at 45 min UV-C and not at 60 min UV-C as in other measurements (Table 2). It has been reported that low UV-C doses trigger photomorphogenic reactions that enhance fruit set, which may be related to light signaling and hormone production of primitive metabolism (Darras et al., 2020). Korkutal et al. (2009) mentioned that UV-C applications on grafted cuttings during the callus period negatively affected shoot development. There is a large body of literature on the biological and physiological effects of UV-B light and the signaling and metabolic pathways it triggers and affects. Much less is known about UV-C light, although it seems to have the potential to be effective in a shorter time than UV-B light (Urban et al. 2016). There are data on the effects of UV-A and UV-B on plant growth in different species, but there are almost no studies describing the effects of UV-C (Yuan et al., 1998; Zhang et al., 2014; Qian et al., 2020; Tevini, 2023).

In order to determine the relationship between the parameters examined in the study, a correlation analysis was also performed (Fig. 6) and as stated by the researchers, TPC level was highly correlated with *trans*-resveratrol and ABTS, while IC<sub>50</sub> was inversely correlated. (Landbo and Meyer, 2001; Kedage et al., 2007). This is because low IC<sub>50</sub> level means high antioxidant activity. From the correlation results, it is understood that high total phenolic compound content indicates high antioxidant activity. In the study, caretonoid and total chlorophyll also have high correlation as stated by Xiao et al. (2017). In addition, the measured enzyme activities are also correlated with IC<sub>50</sub>, indicating that the activities of enzymes increase with antioxidant content under stress factor. Based on the correlated expression of APX and *trans*-resveratrol, it can be concluded that resveratrol may increase the levels of some antioxidant enzymes.

## 5. Conclusion

Considering the limited number of studies on the effects of the C spectrum of UV radiation in viticulture, for the first time, UV-C was applied to the cuttings of grape varieties at different times and its effects were examined in shoots and leaves after rooting the cuttings and it was reported that the parameters examined increased positively as the time increased. Especially the results of 60 min of stress increased total phenolic compounds, *trans*-resveratrol, antioxidant capacity, enzymes and photosynthetic pigments. It was concluded that the 45-minute stress factor was more effective on shoot and root characteristics. This is the first time in the literature that the effect of pre-planting UV-C on shoots and leaves has been reported, and considering that the effects of UV-C may vary depending on dose, duration and frequency, this study will provide preliminary information for future studies. The increase in compounds, which are not only the defense mechanism of plants but also important for our health, is promising. The results of the research give an insight that UV irradiation may be used especially in grapevine nursery by taking advantage of its sterilization properties. It is important to carry out further research to carefully determine the optimal dosage, duration and frequency of UV-C irradiation.

## CRedit authorship contribution statement

**Hande Tahmaz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis,

Data curation, Conceptualization. **Damla Yüksel Küskü:** Investigation, Formal analysis.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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