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Research article

Hormone profile changes occur in roots and leaves of Micro-Tom tomato plants when exposing the aerial part to low doses of UV-B radiation

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ABSTRACT

During the last decades, many studies investigated the effects of UV-B on the above-ground organs of plants, directly reached by the radiation but, to the best of our knowledges, the influence of mild UV-B doses on root hormones was not explored. Consequently, this research aimed at understanding whether low, not-stressful doses of UV-B radiation applied above-ground influenced the hormone concentrations in leaves and roots of Micro-Tom tomato (*Solanum lycopersicum* L.) plants during 11 days of treatment and after 3 days of recovery. In particular, ethylene, abscisic acid, jasmonic acid, salicylic acid and indoleacetic acid were investigated. The unchanged levels of chlorophyll *a and b*, lutein, total xanthophylls and carotenoids, as well as the similar H_2O_2 concentration between control and treated groups suggest that the UV-B dose applied was well tolerated by the plants. Leaf ethylene emission decreased after 8 and 11 days of irradiation, while no effect was found in roots. Conversely, indoleacetic acid underwent a significant reduction in both organs, though in the roots the decrease occurred only at the end of the recovery period. Salicylic acid increased transiently in both leaves and roots on day 8. Changes in leaf and root hormone levels induced by UV-B radiation were not accompanied by marked alterations of plant architecture. The results show that irradiation of above-ground organs with low UV-B doses can affect the hormone concentrations also in roots, with likely implications in stress and acclimation responses mediated by these signal molecules.

1. Introduction

Light plays a key role in the entire life cycle of plants, influencing many morphological, physiological and developmental processes. The wavelength, intensity and duration of the exposure lead to the activation of specific signalling pathways and downstream gene expression, in turn inducing strictly related photomorphogenic responses (Heijde and Ulm, 2012).

Among the different radiations reaching the Earth, the ultraviolet-B one (UV–B, 280–315 nm) became of scientific and public interest in the past decades because of the harmful effects linked to its increased level in the biosphere caused by the thinning of the ozone layer (Andrady et al., 2005; Rowland, 2006). However, nowadays UV-B radiation is studied also from a different perspective: no longer as a plant stressor but as an environmental regulator of plant growth (Coffey et al., 2017), and as a physic tool to improve the nutraceutical qualities and the shelf life of fruits and vegetables (Castagna et al., 2014; Scattino et al., 2016; Santin et al., 2018; Mosadegh et al., 2018). Plants can perceive different light wavelengths by several specific photoreceptors which allow the fine regulation of the events necessary to adapt to the surrounding environment. Among these, the UV-B specific receptor UVR8 (UV RESISTANCE LOCUS 8) is the most recently discovered photoreceptor (Rizzini et al., 2011). The main genes regulated by UVR8 are related to morphological changes, antioxidant protection and defence (Hideg et al., 2013). Some of the renowned plant responses to UV-B include the induction of phenolic compounds which play a role as antioxidants and act similarly to natural sunscreens (Hideg et al., 2013). In addition, changes in the plant architecture - among which leaf shape, alteration of the root to shoot ratio and decrease of stem elongation - also occur under UV-B light (Jansen, 2002; Robson et al., 2015). However, the role of UVR8 in some of these processes has yet to be clarified. Indeed, the non-UVR8 signalling pathway can be stimulated under natural high UV-B levels in non-acclimated plants, causing the upregulation of genes involved in the response to generic stresses (Robson et al., 2015).

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However, it is difficult to generalize the effects of UV-B radiation on the physiology of plants since different experimental acclimation conditions affect the results. Moreover, not all plant species behave in the same way, demonstrating different tolerance thresholds towards UV-B (Jansen, 2002). Experimental designs based on low doses of longer UV-B supplemental radiation allow studying the photomorphogenic modifications of plants specifically regulated by UVR8 (Jenkins, 2017; Favory et al., 2009). On the contrary, higher doses of shorter UV-B wavelengths are likely to induce the expression of sets of genes shared with other stress pathways (Ulm et al., 2004; Brown and Jenkins, 2008). Generation of reactive oxygen species (ROS) as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) may occur in response to UV-B radiation, though the accumulation at harmful levels seems to be restricted to high exposure levels (Czégény et al., 2016). Among ROS, H_2O_2 deserves a particular interest due to its dual role as a pro-oxidant species and as a component of the signal transmission pathway.

Hormones such as auxins, ethylene (ET), gibberellins (GA) abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and cytokinins are deeply involved in the regulation of the morphological and metabolic responses in plants. Evidences exist on the influence of UV-B radiation on the hormonal pathways and downstream effects on plant morphology as well as on the defensive mechanisms in relation to the plant species and/or the dose applied (Vanhaelewyn et al., 2016). Auxins, cytokinins and GA are growth-promoting molecules, ABA, SA and JA are primarily involved in stress response and adaptation and may inhibit plant growth, while ET is a gaseous hormone that affects both morphogenesis and stress response.

Most studies concerning the hormonal response to UV-B focused on the above-ground organs, reporting a positive effect of UV-B radiation on stress-associated hormones (ABA, JA and SA). In contrast, UV-B is reported to inhibit those hormonal pathways known to play a central role in plant morphogenesis (auxins, GA), while ET behaves differently depending on the UV-B doses (Vanhaelewyn et al., 2016 and references within). However, UV-B is known to influence root morphology as well (Robson et al., 2015), suggesting a perceiving mechanism also in the roots and/or a shoot-to-root signalling transmission. Roots are equipped with the same photoreceptors present in other organs and *Arabidopsis* roots also express the UVR8 photoreceptor and specific regulators (Tong et al., 2008; Leasure et al., 2009), suggesting the ability to actively respond to UV-B radiation.

A recent work by Zhang et al. (2019) investigated the interaction among some hormones and root growth and morphology in soybean (*Glycine max* L.) under high UV-B radiation, simulating the UV-B increase under O_3 layer depletion. These authors observed a decrease of some growth-promoting hormones and an increase in the levels of growth-inhibiting ones. However, their results are likely related to stress conditions caused by the high UV-B dose applied, as also suggested by the increase in the hydrogen peroxide and nitric oxide levels.

To the best of our knowledges, there are no reports on the effects of mild UV-B doses on root hormones and signalling molecules. Thus, the present research was focused to understand whether low doses of UV-B radiation were effective in determining a hormonal response also in the below-ground (roots) organs and whether such response was similar to the leaf one. Indeed, there is still little understanding of the effects of UV-B on root hormones despite root growth and morphology, as well as their reactions to stress, are sensitive to light. For this purpose, the level of hormones that are mainly associated with stress such as ET, ABA, JA and SA, and IAA, and are also involved in acclimation processes under moderate UV-B dose, were investigated in both roots and leaves of Micro-Tom tomato (*Solanum lycopersicum* L.) subjected to daily UV-B irradiation for 11 days. Recently, the scientific community involved in UV-plant interactions highlighted the importance of going beyond the classic *Arabidopsis* model plant. Being tomato one of the most important crop species worldwide, the results of the hormonal response to UV radiation, besides being of general interest for basic research, could potentially have an applicative impact. Specifically, in this study Micro-Tom tomato has been chosen as plant model as it is a bush-type tomato easy to be managed in growth chamber conditions. To ensure that the UV-B doses applied did not induce an oxidative stress that could hide the responses triggered by the specific UVR8-mediated pathway, photochemical efficiency, photosynthetic pigments, H_2O_2 accumulation, lipid peroxidation and phenolic and flavonoid concentrations of leaves and roots were determined. Leaf and root biometric parameters were also measured to check possible relationships between UV-B-induced changes in hormone levels and alteration of plant growth/architecture.

2. Materials and methods

2.1. Plant cultivation and UV-B exposure

Seeds of *Solanum lycopersicum* L. cultivar Micro-Tom were purchased from JustSeed Ltd (Wrexham, United Kingdom). Seeds were surface sterilized in a 5% sodium hypochlorite solution for 20 min, washed four times with sterile water and germinated on water-soaked paper. Seedlings were moved in pots containing perlite and, after one week, were transferred to a Hoagland solution (pH~6) in a climate chamber at 24 ± 2 °C, with a 16 h light/8 h dark photoperiod and photosynthetic photon flux density (PPFD) of 228 μmol m^{-2} s^{-1} supplied by blue/red (1:2 ratio) and green (10%) LEDs (C-LED, Imola, Italy). Once a week, the Hoagland solution was completely replaced. Twenty-five-day-old plantlets were divided in two groups: a control group (CTR), grown under PAR radiation only, and a UV-B-treated group (UVB), grown under PAR radiation plus UV-B radiation (15 min a day corresponding to 1.19 kJ m^{-2}) provided by Philips Ultraviolet-B Narrowband lamps (TL 20W/01 - RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands). The irradiance at the top of the canopy was 1.33 W m^{-2}, which is slightly more than the mean daily irradiance peak in Pisa during Summer (Häder et al., 2007). UV-B intensity was quantified by a JAZ EL-XR1 spectroradiometer (OCEAN OPTICS, Dunedin, FL, USA). Leaves and roots of both treated and control groups were collected on the 8th and 11th day of the treatment and 3 days after the end of the treatment.

For each sampling day and treatment three plants were used for analyses. For ET emission, photochemical efficiency and biometric analysis 5 biological replicates were assayed. Each plant represented a single biological replicate and a pool of leaves and the whole root were used for each biological replicate. ET measurement as well as detection of H_2O_2 by the DAB assay were performed on freshly harvested samples, while for all the other biochemical analyses, samples were frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Biometric indexes

All leaves and the whole roots from 5 different biological replicates for each group and sampling day were weighted to obtain the fresh weight (g FW) and then oven-dried to obtain the dry weight (g DW; 50 °C for 1 week). The total number of leaves, the leaf area - determined by a planimeter (Delta-T Device, Cambridge, UK) - and the root length (cm) were also measured.

2.3. Phenol and flavonoid extraction and determination

Frozen leaf and root samples were extracted following the method of Becatti et al. (2010). To determine the total phenol amount in both control and irradiated samples, the Folin-Ciocalteu method (Barbolan et al., 2003) was carried out recording the absorbance at 750 nm by an Ultrospec 2100 pro-UV–vis spectrophotometer (Amersham Biosciences). Total phenols were expressed as μg of gallic acid equivalents g^{-1} FW.

Total flavonoids were determined referring to Kim et al. (2003) recording the absorbance at 510 nm and expressed as μg of catechin equivalents g^{-1} FW.

For both phenol and flavonoid assays, a standard curve was calculated using the corresponding commercial standards (Sigma-Aldrich Chemical Co., St. Louis, MO, USA).

2.4. Antioxidant activity evaluation

The antioxidant activity of the leaf and root phenolic extracts was evaluated by the ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) assay following Pellegrini et al. (1999). The results were expressed as μmol Trolox equivalents g^{-1} FW.

2.5. Chlorophyll a fluorescence

To understand whether the applied dose could affect the photosynthetic process a miniaturized pulse amplitude-modulated fluorometer (Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany) was used for the measurement of chlorophyll *a* fluorescence of control and UV-B-treated leaves. The maximum PSII photochemical efficiency (Fv/Fm), measured after at least 30 min of dark adaptation, and the photochemical yield of PSII in the light (ΦPSII) were measured as described in Huarancca Reyes et al. (2018).

2.6. Chlorophyll and carotenoid determination

Chlorophylls *a and b*, and the carotenoids β-carotene, neoxanthin, lutein, violaxanthin, antheraxanthin and zeaxanthin were extracted and analysed according to Castagna et al. (2013). After filtration, the extracts were run in a Spectra System P4000 HPLC equipped with a UV 6000 LP photodiode array detector (Thermo Fisher Scientific, Waltham, MA, USA) using a Zorbax ODS column (SA, 5-μm particle size, 250 × 4.6 mm; Phenomenex, Castel Maggiore, Italy) with a flow rate of 1 mL min^{-1}. Solvent A, acetonitrile/methanol (75/25), and solvent B, methanol/ethyl acetate (68/32), were used with the following gradient:

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
15	100	0
17.5	0	100
32	0	100
34	100	0
40	100	0

The photosynthetic pigments were detected at 445 nm and data were expressed as μg g^{-1} FW. Commercial standards of chlorophylls and carotenoids (Sigma-Aldrich, Milan, Italy) were used to obtain external calibration curves. The de-epoxidation state of the xanthophyll cycle (DEPS) was calculated as [(A/2) + Z]/(V + A + Z) × 100 (A = anteraxanthin; Z = zeaxanthin; V = violaxanthin).

2.7. H_2O_2 histochemical detection and quantification

Leaf H_2O_2 was histochemically detected by the 3,3′-diaminobenzidine (DAB) assay as reported by Castagna et al. (2007). The first 3 leaves of each plant were collected at the end of the UV-B treatments and vacuum-infiltrated (−60 kPa) with 0.1% DAB in 10 mM MES, pH 6.5 (3 infiltration cycles, 1 min each). After 1 h incubation at room temperature, leaves were boiled at 40 °C in 96% ethanol until complete chlorophyll removal and stored in 50% ethanol. Leaves were observed by both stereomicroscope and light microscope at 100 × magnification and photographed.

H_2O_2 was quantified using the method of Velikova et al. (2000) with slight modifications. Leaf and root samples (0.2 g), previously ground with liquid nitrogen, were mixed in an ice bath with 0.1% trichloroacetic acid for 10 min and then centrifuged at 12.000 × g for 15 min. The supernatant (0.5 mL) was collected and added to a mixture composed by 10 mM potassium phosphate buffer, pH 6.5 (0.5 mL), and 1 M KI (1 mL). The absorbance was read at 390 nm after 1 h of incubation in the dark. Hydrogen peroxide concentration was calculated on the basis of a standard curve prepared with known concentrations of H_2O_2. Data were expressed as nmol g^{-1} FW.

2.8. Lipid peroxidation measurement

Lipid peroxidation was evaluated in leaves and roots by the TBARS (thiobarbituric acid reactive substances) assay based on the method of Hodges et al. (1999) with the following modifications. Leaves and roots were ground in 5% trichloroacetic acid (TCA, 1:10 w/v), centrifuged at 10000 × g for 15 min and the supernatant collected. The extract (200 μL) was added to 1 mL of either -TBA (15% TCA and 0.01% butylated hydroxytoluene) or + TBA (15% TCA, 0.375% TBA, 0.01% butylated hydroxytoluene) solutions. Samples were vigorously shaken, heated at 100 °C in a block heater for 15 min and left to cool down in an ice bath. The absorbances of the extracts were read at 532, 440 and 600 nm, and malondialdehyde equivalents were expressed as nmol g^{-1} FW.

2.9. Hormone extraction and quantification

Leaf and root hormones were quantified on days 8 and 11 of the UV-B treatment and 3 days after the end. Measurements were carried out using a pool of leaves collected from individual plants and the whole root apparatus. Samples were collected immediately after the end of the treatment.

For ET emission, after 10 min from the excision the samples were incubated at room temperature (24 °C) for 1 h into sealed flasks (volume of 30 mL for leaves and 10 mL for roots) equipped with plastic screw caps endowed with a hole and a rubber septum to hallow the collection of ET from the head space through a hypodermic syringe. ET samples (2 mL) were injected into an HP 6890 gas-chromatograph (Hewlett Packard, Milano, Italy) equipped with a dual flame ionization detector and a metal column (150 × 0.4 cm internal diameter) packed with HaySep® T (Agilent Technologies, Milan, Italy). The temperatures of the column and the detector were 70 and 350 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 30 mL min^{-1} (Mensuali Sodi et al., 1992). Data were expressed as pL g^{-1} h^{-1} FW.

Approximately 500 mg of leaves and roots on days 8 and 11 of the UV-B treatment and 3 days after the end were collected for IAA, SA, ABA and JA analyses. The material was homogenized in cold 80% (v/v) methanol (1:5, w/v) using a microdevice as reported by Mariotti et al. (2018). Deuterated [$^2H_4$]-SA, [$^2H_5$]-JA, [$^2H_6$]-ABA (CDN Isotopes Inc., Quebec, Canada) and [$^{13}C_6$]-IAA (Cambridge Isotopes Laboratories Inc., Andover, MA, USA) were added as internal standards to account for purification losses. Methanol was evaporated under vacuum at 35 °C and the aqueous phase was partitioned against ethyl acetate after adjusting the pH to 2.8. The extracts were dried and resuspended in 0.3–0.5 mL of water with 0.01% acetic acid and 10% methanol. HPLC analysis was carried out with a Kontron instrument (Munich, Germany) equipped with a UV absorbance detector operating at 214 nm. The samples, applied to a ODS Hypersil column (150 × 4.6 mm I.D. and 5 μm particle size) (Thermo), were eluted at a flow rate of 1 mL min^{-1}. The column held constant at 10% MeOH for 5 min, followed by a double gradient elution from 10 to 30% and 30–100% over 20 min. The fraction corresponding to the elution volume of SA and IAA was dried and silylated with N,Obis (trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane (Pierce, Rockford, IL, USA) at 70 °C for 1 h, while the fraction corresponding to the elution volume of ABA and JA was dried under vacuum and methylated with ethereal diazomethane. Chromatography-tandem mass spectrometry (GC-MS/MS) analysis was performed on a Saturn 2200 quadrupole ion trap mass spectrometer coupled with a CP-3800 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA, USA) equipped with a MEGA 1MS capillary

column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Mega, Milano, Italy). The carrier gas was helium with a linear speed of 60 cm s^{-1} (the limit detection of the instrument was less than 200 pg). The oven temperature was maintained at 80 °C for 2 min and increased to 300 °C at a rate of 10 °C min^{-1}. The injector and the transfer line were set at 250 °C, and the ion source temperature at 200 °C. Full scan mass spectra were obtained in the EI + mode with an emission current of 10 μA and an axial modulation of 4 V. Data acquisition was from 100 to 600 Da at a speed of 1.4 scan s^{-1}. Hormones were identified by comparison of full mass spectra with those of authentic compounds. Quantification was carried out with reference to standard plots of concentration versus ion ratios, obtained by analysing known mixtures of unlabelled and labelled hormones. Data were expressed as ng g^{-1} FW.

2.10. Statistical analysis

For each investigated day, the differences between control and treated leaves and roots were evaluated by one-way ANOVA using the JMP software (SAS Institute, Inc., Cary, NC). Tukey's test at the 0.05 significance was used for the separation of means. Data represent means ± SE (Standard Error).

3. Results

3.1. Biometric indexes

No significant changes were observed in leaf and root FW and DW during UV-B exposure nor after the withdrawal of the exposure (Table 1). Also leaf total number and root length were not affected by the UV-B treatment (Table 1). However, at the end of the UV-B exposure (11 day) a significant increase in leaf area (+81%) was observed in treated plants (Table 1).

3.2. Phenol and flavonoid concentration and antioxidant activity

Total phenols increased significantly in treated leaves on day 8 (+34%), while on day 11 there was a slight decrease (−8%) compared to the control, that was only transient being no more evident 3 days after the end of the irradiation (Fig. 1). Flavonoid concentration showed a 49% increase in leaves following 8 days of treatment, while on day 11 and 3 days after the end of the irradiation there was no difference between the two groups (Fig. 1). Phenols and flavonoids of roots did not show any response to the UV-B irradiation period (Fig. 1).

A significant increase in the antioxidant activity of treated leaves was detected on day 8 (+35%), which is in accordance with the corresponding phenol increase, while no differences were found on day 11 and 3 days after the end of the irradiation (Fig. 2). Roots did not show any change in the antioxidant activity compared to the control (Fig. 2).

3.3. Chlorophyll a fluorescence

The maximum photochemical efficiency of PSII (Fv/Fm) and the actual PSII efficiency in the light-adapted state (Φ_{PSII}) were measured as markers of a possible UV-B-induced stress at the photosynthetic apparatus. Throughout the investigation period, plant did not show any significant difference for both parameters (Table 2).

3.4. Photosynthetic pigments

The concentration of photosynthetic pigments is reported in Table 2. At each time point, the UV-B treatment did not influence the concentration of both chlorophyll *a and b*. Similarly, no change in lutein, as well as in total xanthophyll and total carotenoid concentration, was induced by the UV-B irradiation. The sum of the three xanthophylls participating in the violaxanthin cycle (V + A + Z) was also unaffected by UV-B exposure, while the de-epoxidation index of treated plants showed a significant decrease during irradiation (−46% and −39% on days 8 and 11, respectively). Such a decrease was transient as 3 days after the end of the irradiation the DEPS of the treated plants recovered the same value of the control.

3.5. Oxidative stress markers: H_2O_2 accumulation and lipid peroxidation

The possible onset of an oxidative stress induced by the UV-B radiation was tested by checking H_2O_2 accumulation in leaves of Micro-Tom plants. H_2O_2 was quantified also in roots to evaluate whether UV-B irradiation of the above-ground portion of the plant could influence the oxidative status of this organ.

The UV-B dose used in this study did not increase leaf H_2O_2 concentration during the 11-day treatment period and not even after 3 days of recovery (Fig. 2). This result was supported by the histochemical visualization following DAB staining. Indeed, the brown spots indicating H_2O_2 accumulation were similarly distributed in both control and treated samples (Fig. 3).

Root H_2O_2 levels were about ten-fold lower than leaf ones. As for the leaves, no significant differences in H_2O_2 accumulation following UV-B treatment were detected in roots. This trend was also evident 3 days after the end of the irradiation (Fig. 2).

The level of lipid peroxidation in leaves was significantly higher in treated plants on day 11 (+18%), while at the beginning of the irradiation and at the end of the recovery period the UV-B treated leaves showed values equal to the control group (Fig. 2). Lipid peroxidation status was unaltered in roots (Fig. 2).

3.6. Hormone concentrations in leaves and roots

To assess the effect of a low UV-B dose on the hormones involved in acclimation processes or in responses to stress conditions, ET, ABA, SA and its conjugated form, IAA and JA were investigated in both leaves and roots.

Table 1
Biometric measurements in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end. Data represent the mean of 5 replicates ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test. N°, numbers; FW, fresh weight; DW, dry weight.

	8 days		11 days		11 + 3 days	
	CTR	UVB	CTR	UVB	CTR	UVB
Leaf number	24.5 ± 0.3	26.5 ± 2.0	34.0 ± 3.5	32.0 ± 2.4	43.3 ± 4.1	43.0 ± 2.6
Leaf area (cm^2)	70.1 ± 6.5	81.2 ± 6.0	70.0 ± 9.5	126.7 ± 10.7**	111.0 ± 17.8	163.9 ± 20.1
Leaf FW (g)	1.5 ± 0.2	1.7 ± 0.2	2.0 ± 0.4	2.6 ± 0.2	2.8 ± 0.5	3.5 ± 0.5
Leaf DW (g)	0.18 ± 0.02	0.19 ± 0.02	0.23 ± 0.04	0.30 ± 0.03	0.31 ± 0.05	0.35 ± 0.06
Root lenght (cm)	42.9 ± 1.9	45.5 ± 3.9	58.6 ± 4.0	54.3 ± 1.5	63.3 ± 7.4	57.5 ± 2.2
Root FW (g)	1.00 ± 0.12	0.89 ± 0.12	1.51 ± 0.34	1.39 ± 0.17	1.97 ± 0.54	1.95 ± 0.43
Root DW (g)	0.06 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.11 ± 0.01	0.10 ± 0.02

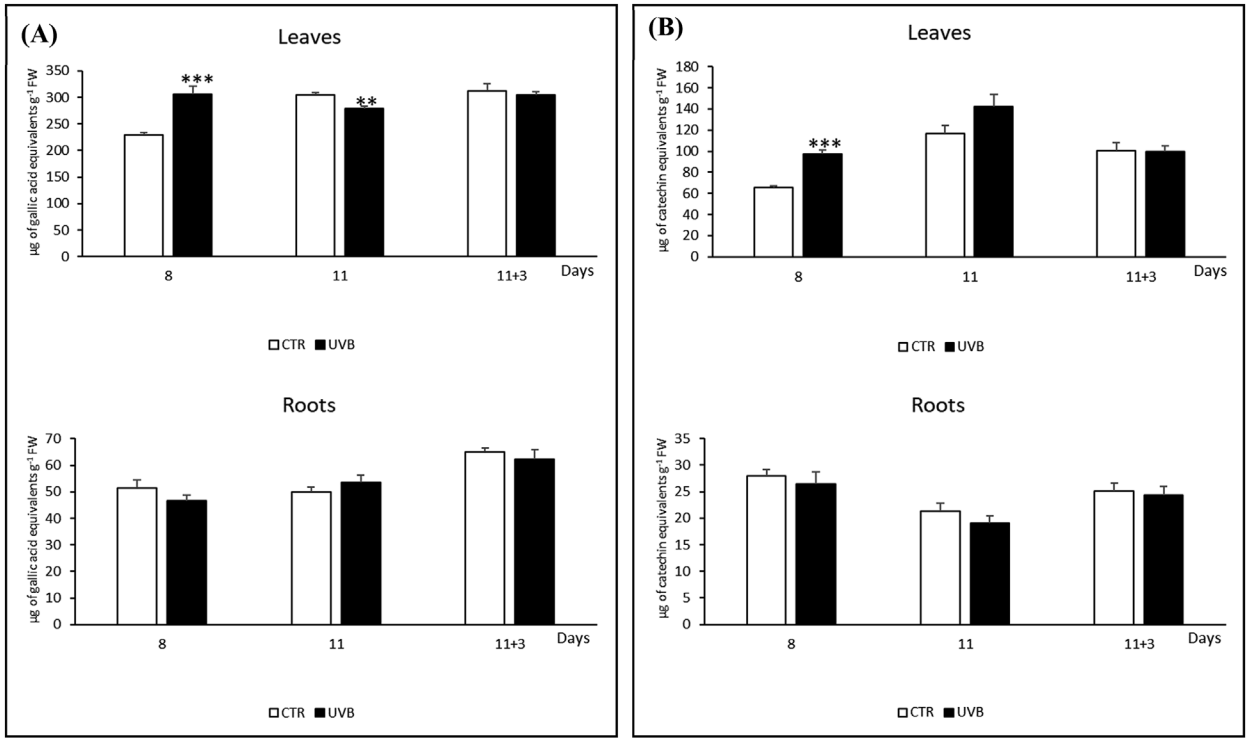

Fig. 1. Leaf and root phenols (µg of gallic acid equivalents·g^{-1} FW) (**A**) and flavonoids concentration (µg of catechin· equivalents g^{-1} FW) (**B**) in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end of the treatment. Data represent the mean of 3 replicates ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test.

ET emission from UV-B-treated leaves underwent a similar significant decrease at both harvesting time points (−35% and −42% on day 8 and 11, respectively; Fig. 4). However, such a decrease was transient since no difference in ET emission was detected 3 days after the end of the UV-B irradiation. Roots exhibited a different behaviour than leaves, ET evolution being unaffected by the UV-B treatment (Fig. 4).

In control leaves IAA concentration showed a progressive increase during the experimental period. A quite different trend was observed in UV-B-treated leaves, which resulted in a marked reduction of the IAA level (- 91%) after 11 days of irradiation compared to the control (Fig. 4). At the end of the recovery period IAA concentration was still much lower than the control (−95%; Fig. 4). Roots exhibited significant differences between control and UV-B groups only 3 days after the end of the treatment, treated plants showing a 60% reduction in IAA level in comparison with the control (Fig. 4).

The influence of UV-B radiation on leaf SA concentration differed during the 11-day irradiation period. In detail, SA level significantly

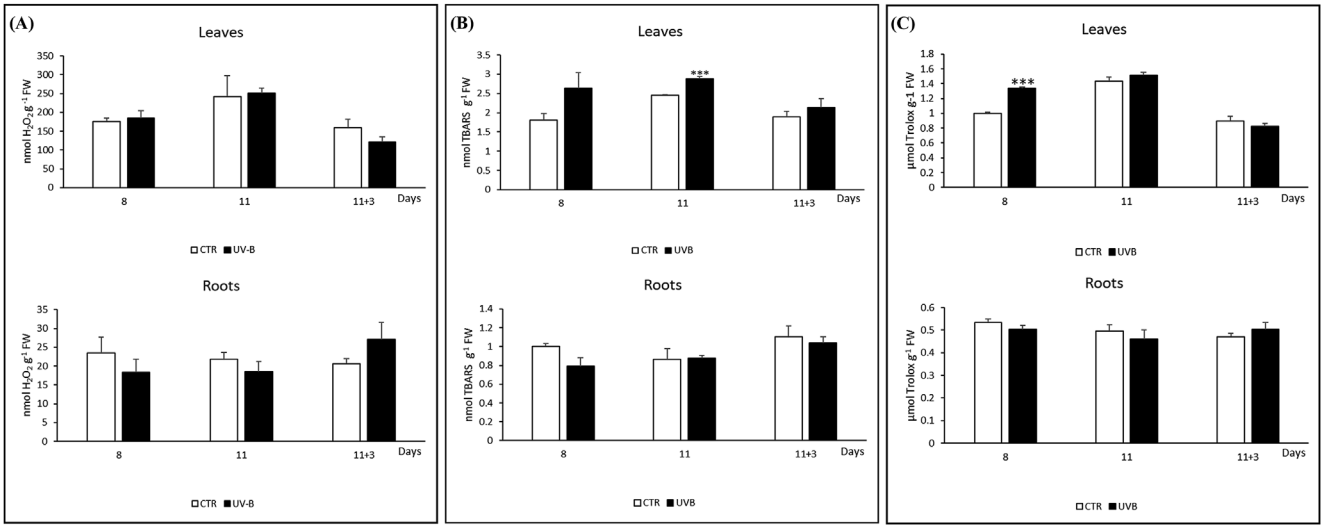

Fig. 2. Leaf and root H_2O_2 concentration (nmol of H_2O_2 ·g^{-1} FW) (**A**), lipid peroxidation (nmol TBARS ·g^{-1} FW) (**B**) and antioxidant activity (µmol of Trolox ·g^{-1} FW) (**C**) in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end of the treatment. Data represent the mean of 3 replicates ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test.

Table 2
Leaf pigments concentration (μg g^{-1} FW) and de-epoxidation index (%), the actual PSII efficiency in the light-adapted state (Φ_{PSII}) and the maximum photochemical efficiency of PSII (Fv/Fm) in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiationand 3 days after the end. Data represent the mean of 3 replicates ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test. V + A + Z, sum of violaxanthin, antheraxanthin and zeaxanthin; DEPS index, de-epoxidation index.

	8 days		11 days		11 + 3 days	
	CTR	UVB	CTR	UVB	CTR	UVB
Chlorophyll *a*	2638 ± 68	3045 ± 331	2312 ± 383	3024 ± 95	2018 ± 350	2681 ± 403
Chlorophyll *b*	523 ± 10	659 ± 79	467 ± 78	608 ± 33	460 ± 85	586 ± 93
Lutein	208 ± 3	250 ± 40	177 ± 29	225 ± 14	207 ± 30	232 ± 28
V + A + Z	135 ± 4	111 ± 18	89 ± 10	110 ± 12	121 ± 13	122 ± 46
β-carotene	181 ± 15	196 ± 21	206 ± 25	214 ± 13	202 ± 11	194 ± 9
Tot xanthophylls	386 ± 2	417 ± 69	300 ± 46	385 ± 31	379 ± 50	411 ± 46
Tot carotenoids	442 ± 3	452 ± 68	333 ± 52	429 ± 28	391 ± 51	445 ± 51
DEPS index	16.3 ± 2.1	8.8 ± 1.3*	14.7 ± 1.5	8.9 ± 0.3*	14.1 ± 2.3	10.9 ± 0.9
ΦPSII	0.694 ± 0.01	0.695 ± 0.01	0.671 ± 0.02	0.711 ± 0.01	0.681 ± 0.01	0.678 ± 0.00
Fv/Fm	0.79 ± 0.00	0.8 ± 0.00	0.798 ± 0.01	0.797 ± 0.00	0.776 ± 0.01	0.786 ± 0.00

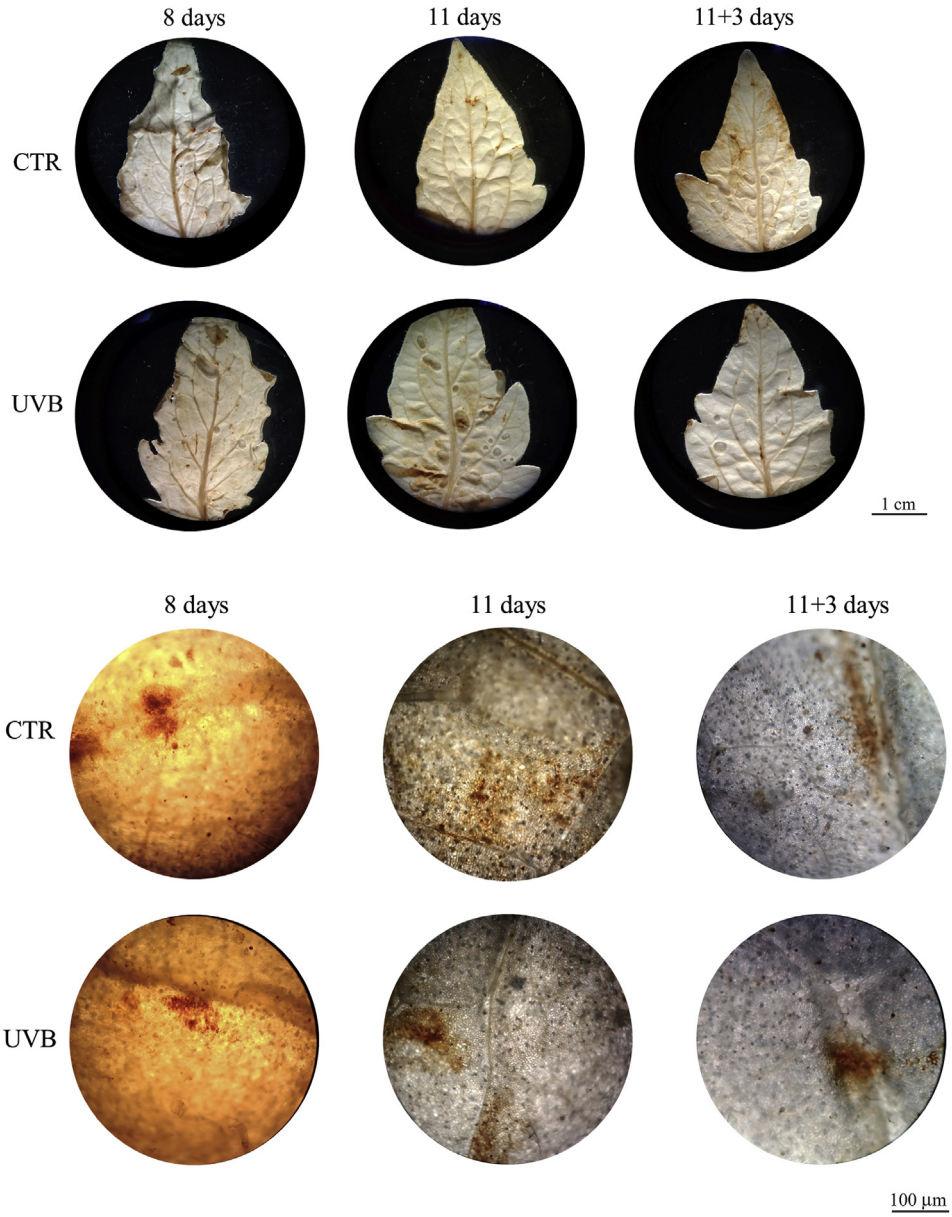

Fig. 3. DAB staining of leaves of untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end of the treatment. The first 3 leaves per plants, 3 biological replicates for control and treated groups, were collected from the end of the UV-B treatment.

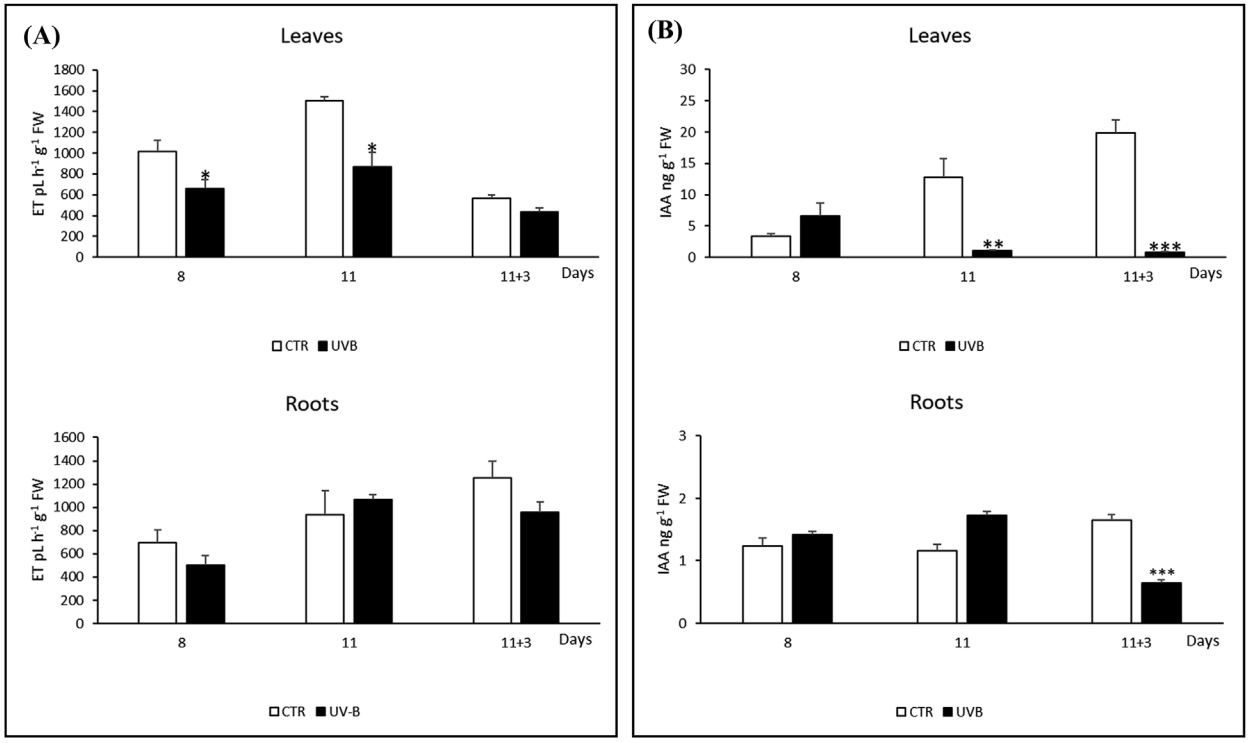

Fig. 4. Leaf and root ethylene emission (ET, $pL \cdot h^{-1} \cdot g^{-1}$ FW) (**A**) and indoleacetic acid concentration (IAA, $ng \cdot g^{-1}$ FW) (**B**) in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end of the treatment. Data represent the mean of 5 replicates for ethylene emission and 3 replicates for IAA ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test.

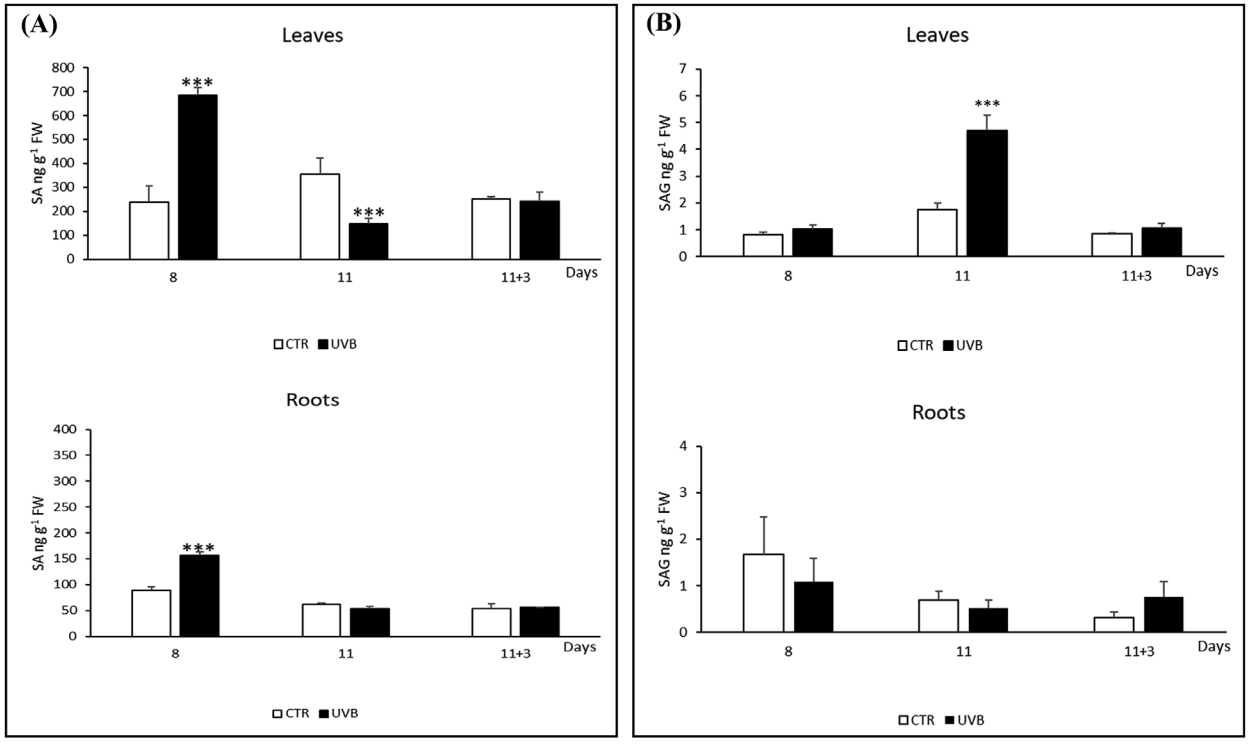

Fig. 5. Leaf and root salicylic acid (SA, $ng \cdot g^{-1}$ FW) (**A**) and SA-glucoside concentration (SAG, $ng \cdot g^{-1}$ FW) (**B**) in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end of the treatment. Data represent the mean of 3 replicates ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test.

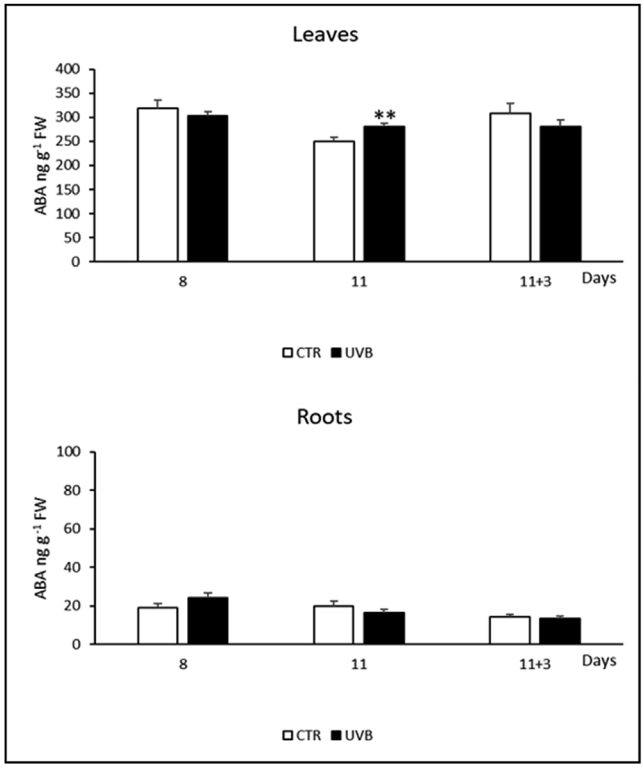

Fig. 6. Leaf and root abscisic acid concentration (ABA, ng $\cdot g^{-1}$ FW) in untreated (CTR) and UV-B-treated Micro-Tom tomato plants at 8, 11 days of irradiation and 3 days after the end of the treatment. Data represent the mean of 3 replicates ± SE. For each day, asterisks (*) indicate significant difference between CTR an UVB group (*P ≤ 0.05, **P < 0.01, ***P < 0.001) according to one-way ANOVA followed by Tukey's test.

increased (+187%) after 8 days of UV-B irradiation (Fig. 5), while at the end of the treatment SA concentration of UV-B-treated leaves was significantly reduced (−58%) compared to the control. Again, as observed for ET, no significant differences were found after 3 days of recovery. The influence of UV-B irradiation was evident also at the root level where, similarly to the leaves, SA concentration showed a significant increase after 8 days of treatment (+77%). However, on day 11 and 3 days after the end, SA levels of both control and treated roots did not differ significantly (Fig. 5).

To better understand the metabolism of SA we also quantified the 2-O-β-D-glucoside (SAG) concentration, which is the main inactive SA conjugate. In UV-B-treated leaves there was a significant enhancement only on the 11th day of irradiation compared to the control, while roots did not show any significant change (Fig. 5).

The influence of UV-B radiation on leaf ABA concentration was not evident after 8 days of irradiation (Fig. 6), while at the end of the treatment (11 days) UV-B-treated leaves showed a slight significant increase in the ABA level (+12%). Similarly to the ET behaviour, the variation in ABA concentration was transient as, once UV-B irradiation was removed, treated and control leaves had similar ABA levels (Fig. 6). As observed for ET, root ABA concentration was not modified by the application of UV-B on the above-ground organs, both during and after irradiation (Fig. 6).

As regards JA, while the level of deuterated JA, added to account for purification losses, was detected in all investigated samples, the endogenous JA resulted under the detection limit of the instrument (0.2 ng) in all samples.

4. Discussion

4.1. UV-B acclimation in Micro-Tom tomato plants

Our first focus was to verify the general health status of UV-B-treated Micro-Tom tomato plants in comparison with a not irradiated control group. This is a key point to ensure that the UV-B dose chosen in this study can be considered as an “eustressor”, namely a positive stimulus that enables plants to acclimate to the new environment. Though stress-related (non-specific) responses and UVR8-mediated signalling can overlap, low UV-B doses are known to preferentially elicit photomorphogenic responses, protective mechanisms and acclimation (Jenkins, 2017).

The unchanged levels of chlorophylls and carotenoids (Table 2) in UV-B-treated leaves suggest that the UV-B dose used was below the stress-inducing threshold. Such hypothesis is supported by a decrease of the DEPS of the xanthophyll cycle (Table 2), which indicates that the excitation pressure on PSII was even lower than in control plants. However, a reduced DEPS value was also reported under more stressful conditions and attributed to a reduced pH gradient across thylakoids due to an altered cyclic electron flow favouring zeaxanthin epoxidation (Guidi et al., 2016). As a confirmation of the good conditions of the photosynthetic apparatus, the maximum photochemical efficiency of PSII (Fv/Fm) and the actual PSII efficiency in the light-adapted state (Φ_{PSII}) showed no differences between control and treated plants during both the UV-B treatment and at the end of the recovery period.

The increase in leaf total phenols and flavonoids (Fig. 1) and in the antioxidant activity (Fig. 2) detected after 8 days of UV-B irradiation is in accordance with the scientific literature, which frequently reported a stimulation of the phenol biosynthesis by this wavelength (Mosadegh et al., 2018; Hectors et al., 2012). A little bit surprising is the lower phenolic concentration observed in the UV-B-treated leaves on day 11. This could result from their oxidation in reactions aimed to maintain ROS below a toxicity level, as suggested by the similar H_2O_2 accumulation detected in both control and treated samples (Figs. 2 and 3). Moreover, soluble phenolics may have been cross-linked to the cell wall by peroxidase-mediated reactions or may have contributed to lignification, thus lowering the soluble phenolic level. Despite this slight reduction on day 11 of UV-B exposure, the antioxidant activity of leaves was unchanged (Fig. 2). The absence in the roots of any significant change in phenols and flavonoids, as well as in the antioxidant activity, suggests that the radiation applied to the above-ground part of the plant was not able to stimulate their biosynthesis in this organ (Figs. 1 and 2).

ROS accumulation is an undoubtful sign of oxidative stress. The lack of differences in H_2O_2 concentrations and in DAB staining (Figs. 2 and 3) between control and UV-B-treated leaves confirms that the dose applied was well tolerated by tomato plants. Our finding is in accordance with the study of Mariz-Ponte et al. (2018), in which a mild UV-B dose (2 min per day for one month, corresponding to 0.353 kJ m^{-2} d^{-1}) did not influence H_2O_2 levels in leaves of Micro-Tom tomato plants. In our study, the same behaviour observed in the leaves was also detected in roots (Fig. 2), confirming that oxidative stress did not play any significant role in the UV-B response of roots.

Despite some signs of lipid peroxidation on the 11th day of treatment (Fig. 2), it is worth noting that the amount of peroxidised lipids was negligible (being less than 3 nmol g^{-1}), lower than the levels detected in control tomato plants by Djebali et al. (2008) during a research on cadmium stress. Moreover, this oxidative indicator did not cause any decrease in the activity of the photosynthetic apparatus or pigment concentration, meaning that the UV-B treatment did not induce any serious damage to the plant. On the 3rd day after the end of the UV-B irradiation there was an evident decrease of the oxidized lipids concentration, meaning that the plant was able to recover the initial status.

All these results confirm a general healthy status of the Micro-Tom tomato plants and their acclimation under the UV-B conditions applied.

4.2. Hormone responses to mild UV-B radiation in roots and leaves of Micro-Tom plants

The core of this research was to investigate whether the hormone profile, in particular that of roots which were hidden from the UV-B radiation, could be modified by this factor and whether root response could be similar to the leaf one. Indeed, there is still little understanding of the effects of UV-B on root hormones, despite root growth and morphology, as well as their reactions to stress, are sensitive to light (Yokawa et al., 2014; van Gelderen et al., 2018).

According to the results of the oxidative stress markers and the photochemical efficiency of PSII, the UV-B dose used was below the stress threshold and likely mainly triggered the UVR8-mediated responses rather than the stress signalling pathway. The reduced ET emission found in UV-B leaves on days 8 and 11 (Fig. 4) agrees with the results of Hectors et al. (2007) who showed in *A. thaliana* a general down-regulation of ET biosynthetic genes *A. thaliana* under mild UV-B radiation and suggests an unlikely involvement of UVR8 in promoting ET biosynthesis. Our results also confirm the evidences reviewed by Vanhaelewyn et al. (2016) that leaf ET production is stimulated by UV-B following exposure to high UV-B intensities, but it is repressed when the UV-B exposure is within photomorphogenic levels. Such a reduction was not evident at the root level (Fig. 4), suggesting that low doses of UV-B are probably able to modify the ET biosynthetic pathway only in the organs directly exposed to the radiation. To the best of our knowledge, ET emission from roots of UV-B treated plants was not investigated so far.

ET is known to influence plant growth by promoting auxin synthesis and controlling its distribution (Vaseva et al., 2018). The decrease of leaf ET emission observed during exposure to UV-B radiation is consistent with the marked reduction in IAA levels detected at the end of both the treatment and the recovery period (Fig. 4). A decrease of IAA concentration induced by a low UV-B dose was also found by Hectors et al. (2012) in young leaves and apex of *A. thaliana*. The UVR8 pathway is known to inhibit the genes linked to auxin biosynthesis and signalling (Jenkins, 2017), and many studies point to HY5 as a negative regulator of IAA pathway, for both signalling and transport (Hayes et al., 2014; Sibout et al., 2006; Vanhaelewyn et al., 2016). The auxin accumulates in the roots by local biosynthesis in the stem cells and following phloematic transport from the shoot-synthesizing sites (van Gelderen et al., 2018; Overvoorde et al., 2010). The reduction of the IAA levels detected in UV-B-treated roots during the recovery period (Fig. 4) could be ascribed to a lower IAA basipetal transport, consequent to the decreased production at the leaf level. However, though a direct inhibition of root biosynthesis could not be excluded without a gene expression analysis. A study on the UV-B effects on soybean roots (Zhang et al., 2019) showed a similar decrease of IAA content but, differently from our experiment, such a decrease was observed not only in the recovery period but already during the 5 days of UV-B irradiation. This difference could be ascribed to the higher UV-B doses used by Zhang et al. (2019), who applied the supplemental UV-B radiation (2.63 or 6.17 kJ m^{-2} d^{-1}) on seedlings that were already receiving ambient radiation (7.6 kJ m^{-2} d^{-1}). Consistent with these high UV-B doses, seedlings probably experienced stress conditions, as shown by the increased H_2O_2 and NO levels in the treated roots. However, according to Hectors et al. (2007), auxins seem to be crucial in the response to both acute and chronic UV-B exposure, though the first seems to affect only the hormone distribution (Ulm et al., 2004), while the latter impacts on both auxin synthesis or distribution. Independently from the mechanism responsible for root IAA decrease, the reduced hormone level detected in the recovery period could impact later on root development.

An increase in salicylic acid is usually linked to a positive enhancement of plant defence. In our study SA exhibited a transient increment (day 8) in both treated leaves and roots (Fig. 5). The enhancement of SA under UV-B radiation has been reported in many studies, in particular under high doses of UV-B (Zhang et al., 2019; Bandurska and Cieślak, 2013; Kovács et al., 2014). However, Mewis et al. (2012) found that in broccoli sprouts SA signalling was also activated by low UV-B doses and that pathogenesis-related proteins-1 and -2 homologs, that in *Arabidopsis* are associated with SA pathways, were induced. On day 11, the significant decrease of leaf SA concentration suggests a partial conversion into conjugated forms such as SA-glucoside (Fig. 5) or other forms. The conversion of SA into its glucoside in the cytosol is considered a mechanism activated by the plant to prevent possible damages. SAG can then be transported into the vacuole as an inactive pool to be converted back when necessary (Hennig et al., 1993; Dean and Mills, 2004; Dean et al., 2005). Methylated-SA seems to be the mobile form that can move along the phloem (Park et al., 2007).

This exchange between SA and SAG was not observed in the roots of Micro-Tom tomato. However, also in this organ the increase in SA level was transient (8 days of irradiation, Fig. 5), while a marked enhancement of root SA concentration was detected in soybean after the withdrawal of high UV-B doses (Zhang et al., 2019).

SA is known to interfere with IAA responses. Indeed, Wang et al. (2007) showed that *Arabidopsis* plants subjected to a high SA level displayed phenotypes similar to auxin-deficient or insensitive mutants and demonstrated that this molecule is able to stabilize repressors of the IAA response. On this basis, it could be hypothesized that in our experiment the SA increment could have played a role in reducing IAA concentration. However, the role of SA-IAA interplay in roots needs more researches. Indeed, a recent study on *Arabidopsis* root development by Pasternak et al. (2019) showed that an exogenous SA treatment lower than 50 μM could lead to the accumulation of IAA in the roots, as if this hormone under certain level could act as a developmental regulator, while at higher concentrations it could be involved in the stress responses, among which IAA depletion.

In accordance with the evidences that the UV-B dose used in this research was probably below the stress threshold, the endogenous levels of the stress-related hormone JA were under the detection limit in both leaves and roots. Indeed, the enhancement of JA was observed in case of high UV-B intensities as reported by Mackerness et al. (1999) in *Arabidopsis* and Zhang et al. (2019) in roots of soybean seedlings. The absence of a detectable induction of JA production in leaves and roots of treated tomato plants argues in favour of the absence of stress conditions. However, SA and JA pathways are known to share a complex network. SA can indeed counteract JA signalling pathway (Caarls et al., 2015), for example inducing the degradation of transcription factors such as ORA59 (Pieterse et al., 2012; Van der Does et al., 2013). Thus, the increased SA level in Micro-Tom-treated leaves and roots on day 8 could be, at least partially, responsible for the lack of detectable JA amounts under UV-B radiation.

ABA has been reported to have a protective role against many abiotic stresses such as drought or high salinity (Finkelstein, 2013). At first sight the slight and transient increase in leaf ABA detected after 11 days of irradiation (Fig. 6) might be interpreted as an UV-B stress response, as reported in various species under moderate and high UV-B doses (Pan et al., 2014; Tossi et al., 2009; Esringu et al., 2016). However, it should be noted that the ABA concentration in UV-B-treated leaves is similar to that of control plants on day 8 and at the end of the recovery period. Moreover, the transient character of this change suggests a prompt recover. ABA concentration in Micro-Tom roots was not altered by the treatment, meaning that its biosynthesis or transport was not affected in this organ (Fig. 6). On the contrary, under likely more stressful UV-B conditions than ours, Zhang et al. (2019) showed that in roots of soybean seedlings ABA remained at high levels also after the removal of the UV-B irradiation.

4.3. Biometric analyses

To understand whether changes in the hormone profile induced by UV-B irradiation could affect the plant growth, we carried out some basic biometric measurements (Table 1). The lack of differences in fresh

and dry weights of both organs as well in leaf number and root total length suggests that the UV-B dose was not able to markedly alter the plant architecture. However, a deep investigation of root architecture, area and lateral root growth could provide a more exhaustive knowledge on the effects induced by the reduced IAA and increased SA levels detected in roots. The enhancement of the leaf area after 11 days of UV-B irradiation was surprising as many studies reported a negative influence of UV-B on this parameter (Dotto and Casati, 2017; Hectors et al., 2007). However, as shown by Robson et al. (2015), the UV effects on leaf area are more complex. Indeed, once the UV-B defence was activated and the plants acclimated to the new environment, the break in the leaf development could be overcome resulting in a restoring or even in a compensatory effect, leading to a higher cell enlargement to compensate the reduced cell division. Moreover, Coffey et al. (2017) showed that in outdoor conditions the influence of UV-B on the morphology of *Arabidopsis thaliana* is restricted to the summer, and it is independent of the UVR8-related pathway. From this, we can assume that the typical aspect of plants under UV-B radiation, reported in many scientific papers, could be associated to high intensities but not necessarily to mild and short irradiations.

5. Conclusions

Despite the effects of UV-B radiation on root growth and morphology, as well as the light sensitivity of this organ, have been previously faced, few studies investigated the impact of mild UV-B radiation on root hormones and compared their response to the leaf ones.

This research provides evidence that mild daily UV-B irradiation influences the hormone balance of Micro-Tom tomato plants not only at the leaf level but also in the roots, although this organ was not directly treated with the UV-B radiation. Changes in hormone levels did not negatively affect leaf or root growth, though it cannot be excluded that the decrease of IAA levels detected at the end of the recovery period could impact later on plant development. The reduced ET and IAA levels, together with the response of some oxidative markers, suggest that tomato plants acclimated to low UV-B doses activating the UVR8-mediated responses rather than the stress signalling pathway. Additional specific experiments, e.g. on transcription of UVR8 target genes in the roots of plants exposed to UV-B as well as on HY5 organ-to-organ movement, could help unravelling the involvement of a direct UV-B perception by roots or of a signal cascade starting in the shoots.

Author contributions

AR, AC and MFQ conceived and designed the experiments. AM and LM performed the analyses and statistics. TR performed the Fv/Fm and ΦPSII measurement. AT and AMS helped in the ethylene analysis and discussion. MS helped in sampling and writing the manuscript. AM, LM and AC wrote the manuscript with inputs from the other authors. AR and MFQ edited the manuscript. All authors read and approved the manuscript.

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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.

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