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In-Vivo In-Vitro Screening of *Ocimum basilicum* L. Ecotypes with Differential UV-B Radiation Sensitivity

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Abstract: Elevated UV-B radiation (UV-B) has been previously reported to affect plant development, physiology, and promote the biosynthesis of UV-absorbing compounds. Sweet basil (*Ocimum basilicum* L.) is an aromatic herb, widely cultivated worldwide for its use in the food, pharmaceuticals, and cosmetics industry. This species exhibits high diversity among different ecotypes based on their geographical locations. There has been little research on intra-specific photosynthetic and metabolic differences in UV-B tolerance across ecotypes from different geographical areas. This study evaluated the protection responses to high UV-B radiation of nine *O. basilicum* accessions with different geographic origins. Specifically, the changes in chlorophyll *a* fluorescence parameters and the leaf rosmarinic acid (RA) compound were assessed using an "in vivo-vitro system" in a closed-type plant production system. Our results revealed a significant variation in UV-B protection mechanisms among accessions when plants were treated with high UV-B doses. The accumulation of RA increased significantly by UV-B light treatment in OCI142, OCI148, OCI30, OCI160, and OCI102, with the highest concentration measured in OCI160 plants. This ecotype showed the highest value of the Fv/Fm ratio, 0.70, after 48 h. Recovery of leaf functionality was more rapid in OCI160 than in other sweet basil accessions, which may indicate better photosynthetic capacity associated with enhanced biosynthesis of UV absorbing compounds. This study shows that the biosynthesis of the UV-absorbing compound (RA) represents an effective mechanism to reduce the photoinhibitory and photooxidative damage caused by high UV stress.

Keywords: UV-B stress; photosynthetic performance; UV-B screening compounds; basil; tissue culture

1. Introduction

High ultraviolet B (UV-B wavelengths 280–315 nm) irradiation induces a variety of morphological responses in plants [1,2] and damaging effects on photosynthetic organisms. Damage to DNA, proteins, membranes, chloroplasts, massive generation of reactive oxygen species (ROS) can further lead to impairment of photosynthetic apparatus and induce programmed cell death (PCD), and ultimately the death of the photosynthetic organism [3–5].

Plants have evolved several mechanisms to cope with the detrimental effects of UV-B radiation including filtering UV radiation, repairing UV-induced DNA damage, and scavenging ROS [6,7]. The most common and powerful protective mechanism against UV-B irradiation is the biosynthesis of UV-absorbing compounds, mainly phenolic compounds, hydroxycinnamate esters, and flavonoids which alleviate the harmful effects of UV-B. These compounds act as UV-screens when they accumulate in the chloroplast mesophilic cells and vacuoles of epidermal cells [1]. In addition to their key role as filtering compounds,

phenols are also known to have complement-independent effects such as scavenging of oxygen free radicals to control the deleterious levels of ROS [8]. Under detrimental environmental stress such as high doses of UVB, the performance of the photosynthetic apparatus of plants can be disrupted [9]. For the evaluation of plants' physiological status under stressful conditions, chlorophyll *a* fluorescence (ChlF) is a fast and reliable indicator of photosynthetic capacity and plant stress level. With this type of measurement, without destroying the tested sample, scientists are able to get information on the efficiency of the photosynthetic process [10,11].

The *Lamiaceae* family, one of the most important and widespread medicinal aromatic plant families, contains about 236 genera and 7136 species. Some of the largest genera in this family are *Stachys* (300), *Plectranthus* (300), *Salvia* (900), *Scutellaria* (360), *Teucrium* (250), *Thymus* (220), *Hyptis* (280), *Nepeta* (200), and *Vitex* (250) [12]. The phenolic compounds in this family have different medicinal properties such as antioxidant [13,14], anticancer [15], antifungal [16], and antimicrobial [17] activity and have been long considered as a source of valuable natural substances for health care.

One of the main member species of the *Lamiaceae* family is basil (*Ocimum basilicum* L). This species can accumulate high levels of different phenolics, such as flavonoids, phenolic terpenes, and phenolic acids, mainly rosmarinic acid (RA; R-O-caffeoyl-3-4-dihydroxyphenyllactic acid), an ester of caffeic acid, and 3-4 dihydroxy phenyllactic acid with biological activities including antiviral, antibacterial, antioxidant, and anti-inflammatory activity [18,19]. Basil accessions showed variability at the DNA level (genotypes) [20], essential oil composition (chemotypes) [21], and morphology (morpho-types) [22,23]. Basil plants are able to grow in several regions all over the world [23]. There has been little research on intra-specific photosynthetic and metabolic differences in UV-B tolerance across ecotypes from different geographical areas. Therefore, to gain a better understanding of UV-B stress-tolerant responses, this study evaluated the protection responses to high UV-B radiation in nine *O. basilicum* accessions with different geographic origins. The screening of different plant genotypes to highlight responses to abiotic stress conditions or to verify phytochemicals production under different growth conditions can be carried out with various strategies including in vitro plant tissue cultures [8,24]. This technique has many advantages such as standardized methods and complete control of environmental factors. However, only plants grown in vivo permit the detection of stress symptoms. In this paper, we adopt a culture system *in vitro-vivo* [25] where the autotrophic growth of seedlings could allow the advantages of both approaches to be combined.

2. Materials and Methods

2.1. Plant Material, In Vitro Growth Conditions

Seeds of nine different ecotypes of *Ocinum basilicum* L. var. *basilicum* from the north to the south of the world were obtained from GBIS/I (Genebank Information System of the IPK Gatersleben, Germany) (Table 1). Seeds were soaked for 10 min in a solution of 20% sodium hypochlorite plus 0.01% Triton X-I00 (Sigma Aldrich, Milano, Italy) to sterilize the surface efficiently, and then, they were rinsed four times with sterile water. The seeds were plated on Murashige and Skoog [26] media with 3% (w/v) sucrose and 1.2% (w/v) Agar™ (Sigma Aldrich, Milano, Italy), pH 5.7. In order to promote the synchronization of seed germination, the seeds were placed at 4 °C for 48 h. Then the sown plates were transferred to a growth chamber settled with a temperature of 22 °C, under 16 h (100 μmol^{-1} m^{-2} s^{-1}) of light and 8 h of darkness for germination.

2.2. Culture System

Five-day-old seedlings were transferred and grown in a previously developed "in vitro-vivo system" [25,27]. Briefly, basil seedlings were placed in Falcon™ (BD Bioscience, Belgium) tubes including 60 mL of agarized MS medium without growth regulators and sucrose. The tubes were appropriately modified by making two holes on the cylindrical surface that enabled the growth of two plantlets with the root system growing into an

in-vitro medium and the aerial part "in air" to guarantee the effectiveness of UV-B radiation treatment. After transfer, the stems of the plantlets were sealed with sterile plasticine, and the falcon containers were then placed horizontally on the longer side.

Table 1. *O. basilicum* accessions (ecotypes) and their geographical distributions.

O. basilicum Stock No.	Country of Origin (Location)	Coordinate	Elevation above the Sea Level (m)
OCI142	Maldives	N4 E73	8
OCI148	Maldives	N4 E73	8
OCI149	Maldives	N4 E73	8
OCI30	Argentina	N2 W75	1559
OCI102	Romania	N44 E26	83
OCI126	Georgia	N39 E116	49
OCI370	Spain	N40 W3	665
OCI118	Togo	N8 0-1E	481
OCI160	Cuba	N21 W80	41

The seedlings were grown in Falcon containers at a constant temperature of 22 °C for two weeks under light (100 $\mu mol^{-1}\ m^{-2}\ s^{-1}$ provided by L 36W/76 "Cool White" fluorescent tubes, Osram, Munich, Germany). The plants within the growth chamber were arranged randomly on one shelf.

2.3. UV-B Exposure

The UV-B- treatment was carried out in the dark. Plants were positioned in a shielded cabinet equipped with UV-B lamps (UV-B GL20 SE, Sankyo Denki, Tokyo, Japan), whose emission spectrum ranged λ from 280 to 400 nm, resulting in a maximum peak at 325 nm. Then, the plants were subjected to a high UV-B dose of 68 kJ $m^{-2}\ d^{-1}$. This was achieved through continuous exposure to 4 h min d^{-1} of UV. The fluence of UV-B exposure received by the plants during the experiment was measured using a Light Sensor Reader equipped with a UV Sensor (Field Scout™ Spectrum Technologies, Inc. Plainfield, IL, USA). The control plants were maintained under cool white light at 100 $\mu mol\ m^{-2}\ s^{-1}$ provided by fluorescence tubes. After UV-B treatment, the plants were returned to control conditions (constant temperature of 22 °C, 16 h photoperiod with 100 $\mu mol^{-1}\ m^{-2}\ s^{-1}$ white light, 65% RH) for another 72 h.

2.4. Determination of Rosmarinic Acid

The concentration of rosmarinic acid in sweet basil was performed according to Maggini et al. [28] and Kiferle et al. [24] protocols. Briefly, 300 mg FW of frozen tissues were ground in a mortar with 5 mL of extraction solvent (EtOH:H$_2$O:HCl 80:19:1 *v/v*) and sieved into plastic tubes. Samples were sonicated for 1 h and to prevent the heating of the sample, the sonication was carried out on ice. The samples were incubated overnight at −20 °C. Then, the samples were centrifuged at 3000 rpm for 5 min and the collected supernatants were transferred into fresh tubes. The pellet was extracted with 5 mL of fresh solvent and sonicated again, then it was incubated overnight at −20 °C. After centrifugation, the two supernatants were combined for analysis. Chromafil® syringe filters (0.45 µm cellulose mixed esters membrane; 25 mm diameter; Macherey-Nagel, Düren, Germany) were used for the filtration of all extracts prior to HPLC separations.

HPLC grade solvents were used for the analyses and the HPLC equipment (Jasco, Tokyo, Japan) included a PU-2089 four-solvent low-pressure gradient pump and a MD-4010 diode array detector. Analyses were performed using a Macherey–Nagel C18 250/4.6 Nucleodur® 100-5 Isis column equipped with a guard column, at a flow rate of 1 mL min⁻¹. Acetonitrile (solvent A) and aqueous 0.1% phosphoric acid (solvent B) were used for elution, with the following gradient: 0–0.4 min, A 5%; 0.4–10 min, A 5–50%; 10–15 min, A 50–95%; 15–17 min, A 95%; 17–19 min, A 95–5%; followed by 5 min equilibration (A 5%).

The detection wavelength range was 220–450 nm. The analyses were performed at room temperature (23–24 °C), using an injection volume of 20 μL.

Rosmarinic acid was identified by comparing the retention times and absorption spectra obtained at maximum peak with those of analytically pure standard (Phytolab GmbH, Westenbergsgreuth, Germany).

2.5. Chlorophyll a Fluorescence

Measurements of chlorophyll *a* fluorescence were conducted using a portable Handy-PEA chl fluorometer (Hansatech, Kings Lynn, UK). The leaves were first dark acclimated for 30 min at room temperature by fixing leaf clips (Hansatech, Kings Lynn, UK). Then, chlorophyll *a* fluorescence transients were recorded by illuminating the leaves with a beam of saturating light (3000 μmol m^{-2} s^{-1}) of 650 nm peak wavelength, obtained from three light-emitting diodes focused on the leaf surface through the clips on a spot of 4 mm diameter circle. Leaf fluorescence detection was measured by a fast response PIN photodiode with an RG9 long-pass filter (Hansatech, Kings Lynn, UK, technical manual). JIP analysis was performed to determine the following parameters: (i) Efficiency: maximum quantum yield of primary PSII photochemistry (Fv/Fm); (ii) Specific fluxes per reaction center (RC): the absorption/antenna size (ABS/RC), maximum trapping (TR_0/RC), electrons transferred (ET_0/RC), and dissipated (DI_0/RC) per active PSII; (iii) Phenomenological energy fluxes: the absorption (ABS/CS_0), maximum trapping (TR_0/CS_0), electron fluxes transferred (ET_0/CS_0), dissipation (DI_0/CS_0) and active reaction centers (RC/CS_0) per cross section (CS_0).

2.6. Statistical Analysis

The whole experiment counting the treatment and control was independently repeated three times. The chlorophyll *a* fluorescence measurement was performed immediately after UV-B exposure (0 h), and after 24 and 48 h of recovery time. Rosmarinic acid content was determined at the end of the recovery time (48 h). Statistical analyses were performed with PRISM 6 software (GraphPad Software, San Diego, CA, USA). All data were expressed as means (n = 5) $\pm$ SE of the number of replicates. Through the two-way ANOVA statistical significance effect of UV-B treatments was examined. Differences between various ecotypes treated with high UV-B doses were determined by using the Tukey posttest ($p < 0.05$).

3. Results

3.1. Visual Effects of UV-B Exposures

Nine *O. basilicum* ecotypes accessions originating from different geographical locations were selected to study plant responses to UV radiation. These basil accessions were grown in a previously described "*in vitro-vivo system*" [25,27,29] and were UV-B irradiated using a high UV-B dose in order to depict the overall effects of UV radiation on the magnitude of UV-protection of the photosynthetic machinery using parameters derived from chlorophyll a fluorescence measurements and the accumulation of UV screening phenolic compounds like rosmarinic acid.

The basil plants were irradiated with a high UV-B dose (68 kJ m^{-2} d^{-1}) by continuous exposure to 4 h min d^{-1} of UV, already reported to cause stress on *O. basilicum* plantlets [25,27]. After the UV-B exposure, plants were placed in a light regime along with the already present control plants for up to 48 h (recovery period). Pronounced leaf-browning, necrosis/wilting, and leaf curling symptoms were observed after 48 h of UV-treatment in six accessions (i.e., OCI149, OCI102, OCI118, OCI142, OCI148, OCI126) (Figure 1). On the other hand, glossy leaves and less severe leaf-browning features were observed in OCI370 and OCI30, and noticeably, OCI160 manifested the slightest signs of UV-stress injuries compared to all other ecotypes as shown in Figure 1.
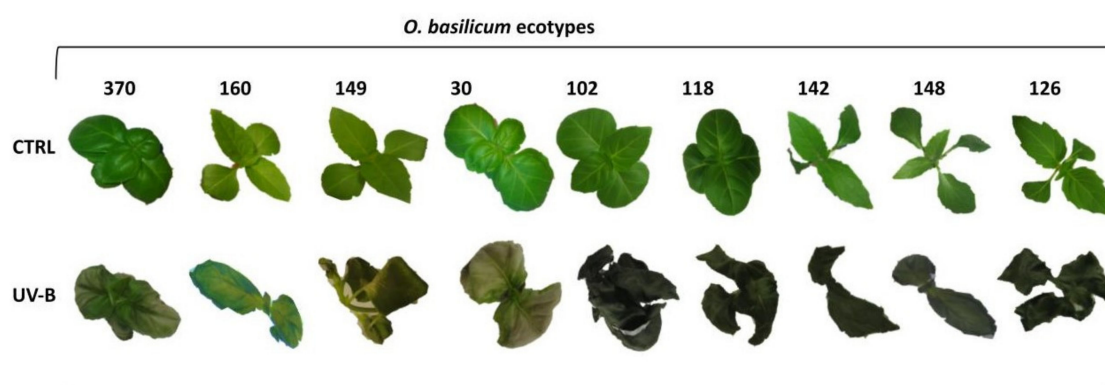
Figure 1. Appearance of nine different *O. basilicum* ecotypes over 48 h, after UV-B exposure (68 kJ m^{-2} day^{-1}) and control (Ctrl) condition (not UV-B exposed).

3.2. Chlorophyll a Fluorescence

The response of different *O. basilicum* accessions subjected to high UV-B dose was evaluated at the level of photosynthetic machinery by measuring the fast chlorophyll *a* fluorescence transient at 0, 4, 24, and 48 h from the beginning of UV-B exposure. The calculated functional parameters linked to the health status of the plantlets, are the specific (per reaction center, RC) and phenomenological (per cross section of the sample, CS) energy fluxes for absorption (ABS), trapping (TR), electron transport (ET), and dissipation (DI) as well as the maximum quantum yield of primary PSII photochemistry (Fv/Fm).

The exposure of basil leaves to high UV-B doses led to a general decrease of the maximum yield of PSII (Fv/Fm ratio) after 4 h (at the end of UV-B light exposure) and it gradually decreased over the 48 h recovery period in almost all basil accessions tested (Table 2). On the other hand, the OCI160 ecotype was the least affected by UV-B light stress and showed the highest Fv/Fm value at 48 h during recovery compared to others (Figure 2).

Table 2. Effect of UV-B exposure (68 kJ m^{-2} day^{-1}) in nine different sweet basil ecotypes at different recovery times (0, 4 h, 24, and 48) on selected chlorophyll *a* fluorescence parameters. Analysis of variance was used for comparisons. Means within each column (*O. basilicum* ecotypes) and within each chlorophyll *a* fluorescence parameter assessed followed by different letters are significantly different ($p < 0.05$; Tukey's multiple range test, n > 5).

|		OCI142	OCI148	OCI149	OCI30	OCI160	OCI370	OCI102	OCI118	OCI126A
		*O. basilicum* Ecotypes								
ABS/RC	0 h	3.32 a	3.28 a	2.77 a	3.24 a	3.41 a	3.47 a	3.25 a	3.36 a	3.29 a
	4 h	4.30 b	4.41 b	5.58 b	4.54 b	3.54 a	4.50 b	4.60 b	5.51 b	4.98 b
	24 h	7.2 c	4.07 b	13.93 c	6.36 b	4.25 b	5.29 cb	14.45 c	4.63 c	10.61 c
	48 h	20.04 d	17.32 c	19.16 d	12.20 d	4.80 c	5.98 c	17.20 d	13.18 d	16.57 d
TRo/RC	0 h	2.73 a	2.81 a	2.92 a	2.91 a	2.87 a	2.83 a	2.85 a	2.74 a	2.71 a
	4 h	2.49 b	2.45 b	2.45 b	2.76 ab	2.72 a	2.80 a	2.68 b	2.44 b	2.34 b
	24 h	2.11 b	2.11 c	2.03 c	2.42 b	2.64 b	2.42 b	2.33 b	2.01 c	1.84 c
	48 h	1.83 c	1.91 d	1.55 d	1.79 c	2.41 c	2.12 c	1.60 c	1.69 d	1.67 d
ETo/RC	0 h	1.59 a	1.38 a	1.56 a	1.55 a	1.48 a	1.47 a	1.38 a	1.56 a	1.43 a
	4 h	1.48 a	1.22 b	1.21 b	1.13 b	1.40 a	1.39 a	1.22 b	1.21 b	0.86 b
	24 h	1.22 b	1.07 c	0.91 c	0.94 c	1.29 b	1.01 b	1.07 c	0.91 c	0.81 b
	48 h	0.40 c	0.38 d	0.62 c	0.68 c	1.07 c	0.79 c	0.38 d	0.62 c	0.59 c
DIo/RC	0 h	0.59 a	0.54 a	0.50 a	0.59 a	0.57 a	0.55 a	0.60 a	0.52 a	0.58 a
	4 h	1.81 b	1.96 b	3.55 b	2.08 b	1.73 b	2.08 b	1.92 b	1.06 b	2.48 b
	24 h	5.37 c	3.17 c	3.67 b	1.93 b	1.82 b	2.83 b	12.11 c	1.57 c	8.94 c
	48 h	17.93 b	15.53 d	19.01 b	12.40 c	2.4 c	3.97 c	20.74 c	16.49 c	18.43 c
RC/CS$_0$	0 h	309.49 a	338.16 a	333.60 a	322.97 a	337.83 a	318.81 a	314.83 a	361.82 a	330.61 a
	4 h	317.62 a	282.03 b	303.69 a	258.04 b	299.57 a	270.30 a	262.83 b	246.09 b	181.67 b
	24 h	230.80 b	277.51 b	172.92 b	242.12 b	255.90 b	201.19 b	187.22 c	150.25 c	155.27 b
	48 h	41.45 c	43.58 c	49.89 c	67.11 c	225.90 c	163.07 c	35.86 d	68.43 d	76.04 c
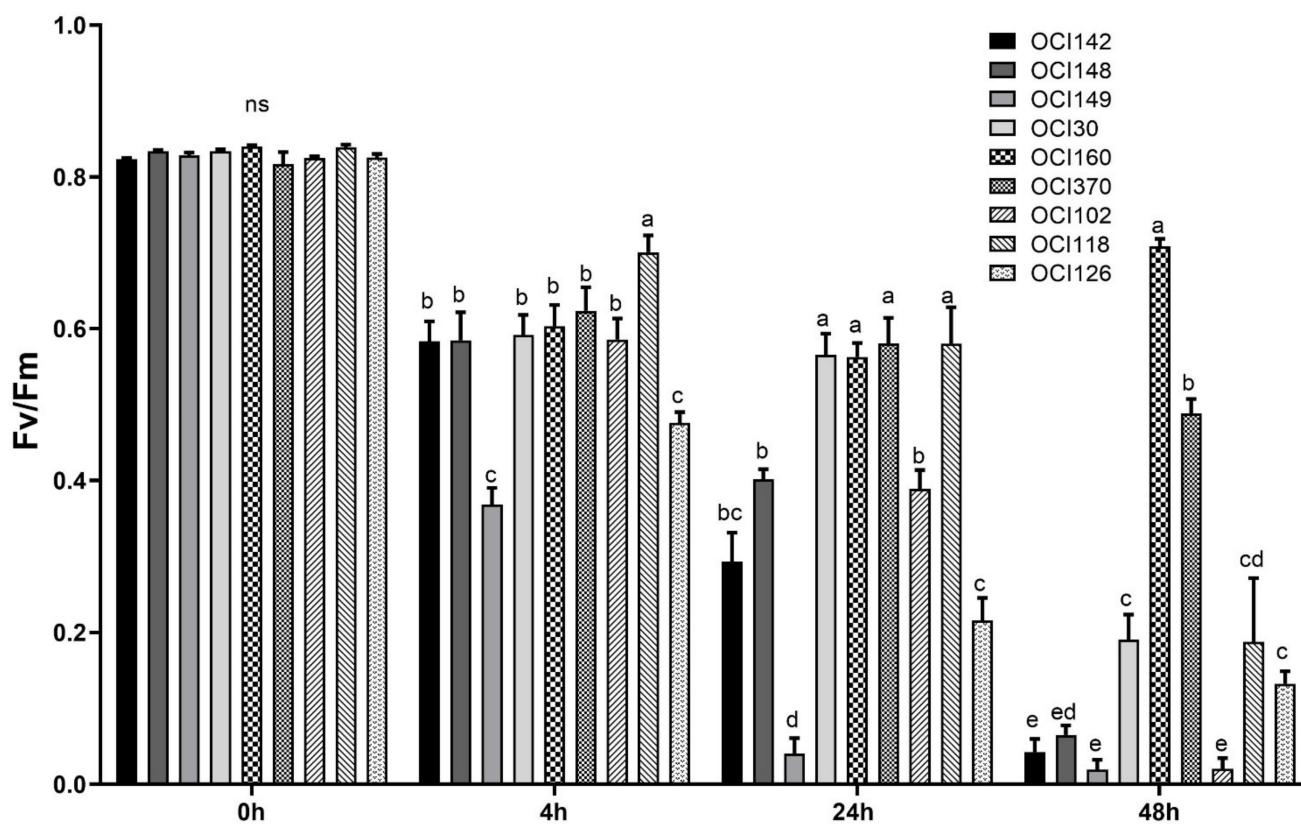
Figure 2. Time course of maximum quantum efficiency of photosystem II, (Fv/Fm) measured in leaves of nine different sweet basil ecotypes exposed to UV-B light (68 kJ m^{-2} day^{-1}) or not exposed (0 h). The measurements were carried out at 0, 4 h, 24 h, and 48 h after the end of the UV-B exposure. Data are shown as means with at least five independent biological replicates and error bars indicate standard error. Data were subjected to analysis of variance and differences were analyzed by Tukey's posttest. Different letters denote significant differences at $p < 0.05$ (ns: not significant).

The parameters related to the specific energy fluxes (ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC) and the phenomenological energy fluxes through the cross section of the sample (CS_0), such as percentage of active reaction centers (RC/CS_0) displayed alterations due to UV-B radiation treatment, as compared to the not UV-B treated ones (0 h) (Table 2). TR_0/RC, ET_0/RC, and RC/CS_0 significantly declined, whereas ABS/CS_0 and DI_0/CS_0 significantly increased with high UV-B radiation in all ecotypes. The lowest decreases in TR_0/RC, ET_0/RC, and RC/CS_0 and the lowest increases in ABS/CS_0 and DI_0/CS_0 were found in OCI160 plants (Table 2).

UV-B radiation caused significant changes in the phenomenological energy fluxes through the cross section of the sample (CS_0) parameters (ABS/CS_0, TR_0/CS_0, ET_0/CS_0, and DI_0/CS_0) (Figure 3).

UV-B was found to increase the values of absorption (ABS)/CS_0 over time in all ecotypes (Figure 3A, Table S1). ABS/CS increased after 48 h of UV-B treatment in OCI160 plants but UV-B radiation caused a less noticeable increase, compared to the others accessions (Figure 3A).

Trapped energy per cross-section (TRo/CS_0) was lower in all UV-B treated ecotypes (Table S1) than in the control plants. A higher value (480 and 334) at the end of the recovery period (48 h) was found in OCI160 and OCI30 plants, respectively, exposed to UV-B light (Figure 3B).
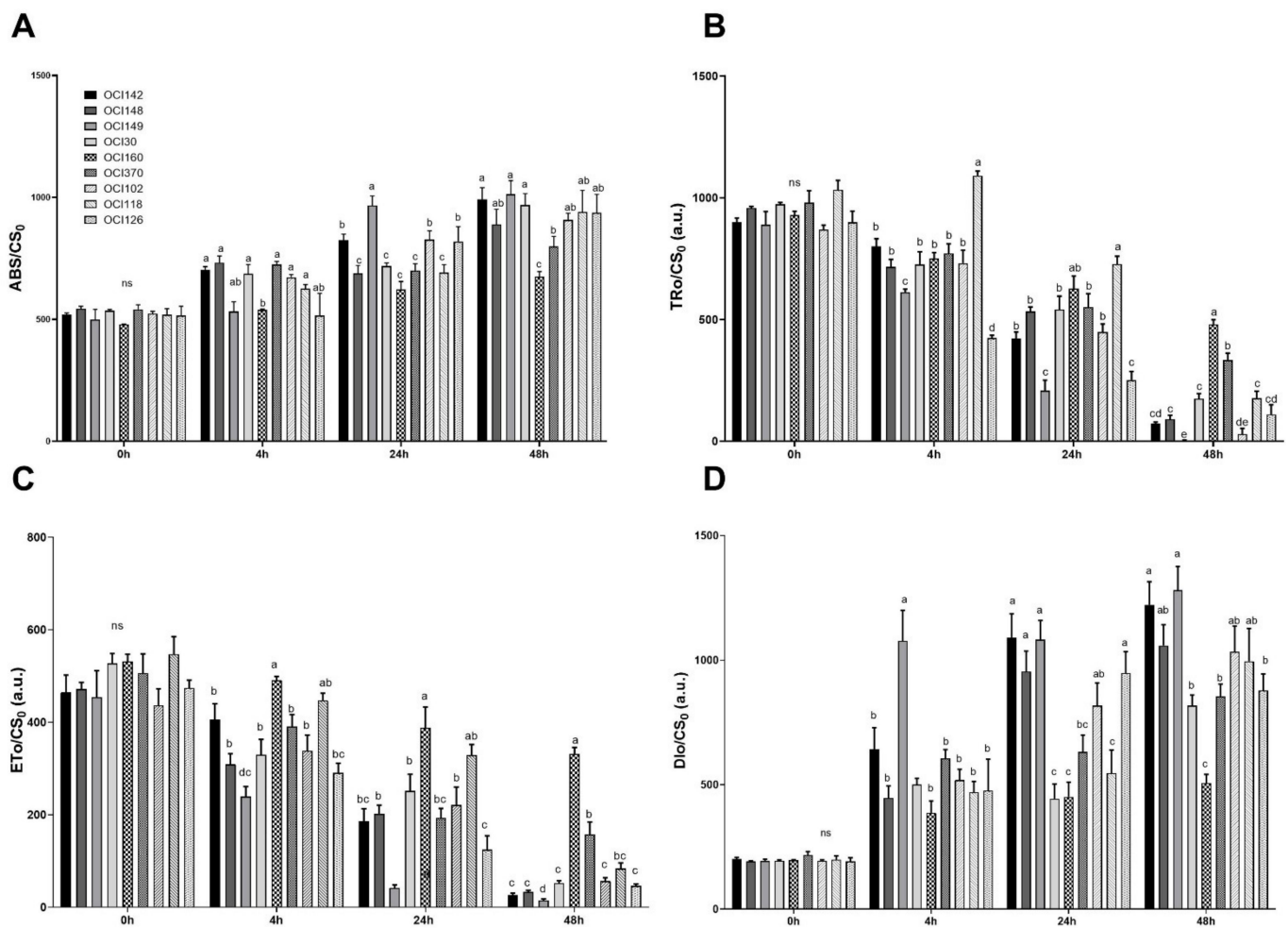
Figure 3. Time course of chlorophyll *a* fluorescence parameters measured in leaves of nine different sweet basil ecotypes exposed to UV-B light (68 kJ m^{-2} day^{-1}) or not exposed (ctrl). (**A**) Absorption flux per cross section, ABS/CS$_0$; (**B**) Trapped energy per cross section, TRo/CS$_0$; (**C**) Electron transport flux per cross section, ETo/CS$_0$; (**D**) Dissipation energy per cross section, DIo/CS$_0$. Data are shown as means with at least five independent biological replicates. The measurements were carried out at 0, 4 h, 24 h, and 48 h after the end of the UV-B exposure. Values are means with standard errors (n = 5) and error bars indicate standard error. Data were subjected to the analysis of variance and differences were analyzed by Tukey's posttest. Different letters within the time denote significant differences at *p* < 0.05.

Similarly, the electron transport flux per cross-section (ETo/CS$_0$) gradually decreased in all ecotypes after high UV-B radiation before drastically dropping after 48 h of UV-B exposure, except for OCI160 exhibiting the highest value at 48 h, followed by OCI30 plants. ETo/CS$_0$ was higher in OCI160 at the end of the experiment, approximately 331, while in all other species the values ranged from 14 to 157 (Table S1, Figure 3C).

The energy dissipated per reaction center (DIo/CS$_0$) in all accessions increased after UV-B exposure (Table S1). However, in OCI160 plants, DIo/CS$_0$ increased less during the 24 and 48 h recovery periods, showing the lowest level compared to the other accessions within the same timing (Figure 3D).

3.3. Effect of High UV-B Light Exposure on the Rosmarinic acid (RA) Content

The concentration of RA varied significantly (*p* < 0.05) among basil accessions grown under high UV-B light after 48 h (Figure 4).
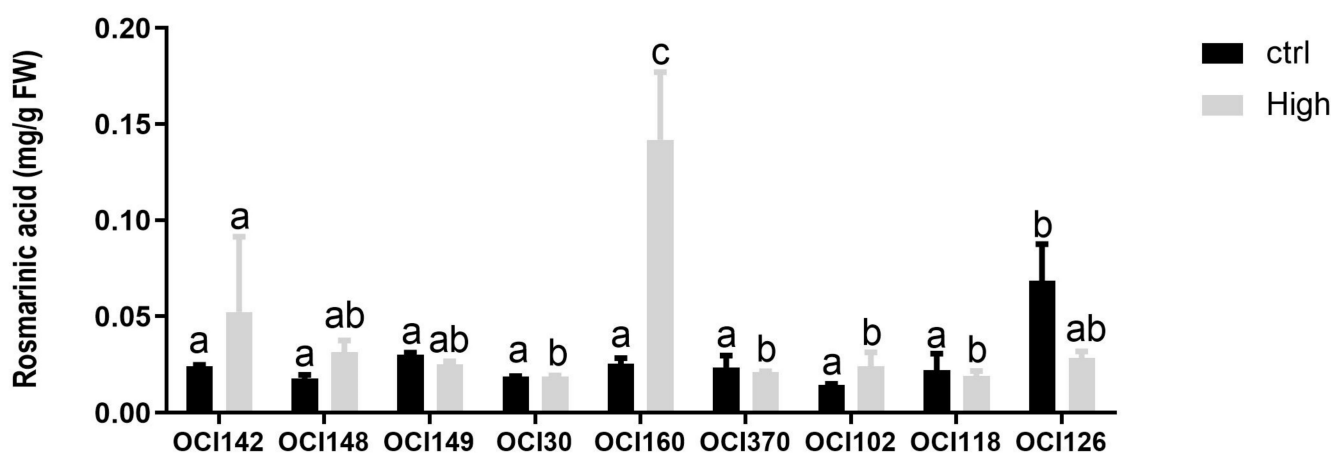
Figure 4. Time course of changes in rosmarinic acid content in leaves of nine different sweet basil ecotypes exposed to UV-B light (68 kJ m^{-2} day^{-1}) or not exposed (ctrl). Data are shown as mean with at least five independent biological replicates. The measurements were carried out at 48 h from the beginning of the exposure. Data were subjected to analysis of variance and differences between ecotypes were analyzed by Tukey's multiple comparisons test. Different letters denote significant differences at $p < 0.05$.

The accumulation of RA increased significantly by UV-B light treatment in OCI142, OCI148, OCI30, OCI160, and OCI102, with the highest concentration measured in OCI160 leaves. In contrast, UV-B exposure reduced the accumulation of RA in OCI102, while the other accessions did not show changes compared to untreated (0 h) plants.

4. Discussion

This study used an "in vitro-in vivo system" in a closed-type plant production system for plant growth in a controlled environment that allowed screening and selection of the highest UV-B tolerant ecotypes of *O. basilicum* by comparing, on the one hand, the biosynthesis/accumulation of a UV-absorbing compound like RA as a protective mechanism to avoid the harmful effects of UV radiation [1] and, on the other side, the effects on the efficiency of PSII photochemistry as the main target of UV-B light-induced inhibition of the photosynthetic machinery [30]. The use of an "in vitro-in vivo system" in a closed-type plant production system, first developed by Srivastava et al. [29] and then modified by Mosadegh et al. [25] allowed the growth of plantlets with root systems growing into the "in-vitro" medium and the aerial part "in the air" to guarantee the effectiveness of UV-B treatment as well as the proper manipulation of UV-B radiation by turning on and off the UV-B lamps. In this screening procedure for ecotypes' UV-B tolerance evaluation, a two-stage specific strategy was applied.

First, the *O. basilicum* ecotypes were grown under control conditions for three weeks and then subjected to UV-B radiation for 4 h reaching a fluence rate of 68 kJ m^{-2} d^{-1} (high UV-B dose), to depict the photosynthetically mediated effects of UV-B radiation using parameters derived from chlorophyll *a* fluorescence measurement, and the changes of UV-inducible RA accumulation in leaf tissue.

The stress-induced damage and adverse effects of high UV-B radiation on photosynthetic activity (mainly related to PSII performance) has been broadly accepted [27,30–32] and chlorophyll *a* fluorescence is used as a non-invasive measurement of PSII activity for rapid screening of photosynthesis that gives high-resolution information regarding a plant's status and thus very useful to analyse the functionality of leaves exposed to several stressful conditions [33–39].

When basil leaves were exposed to high UV-B radiation a gradual decrease of the maximum yield of PSII (Fv/Fm ratio) after 4 h (at the end of UV-B light exposure) and gradually decreased over the 48 h recovery period in almost sweet basil accessions tested was observed (Table S1). On the other hand, the OCI160 ecotype was the least affected by

UV-B light stress and showed the highest Fv/Fm value at 48 h during recovery compared to others (Figure 2).

High UV-B irradiation gradually decreased the maximal photochemical activity of PSII (expressed as Fv/Fm), measured after dark adaptation in sweet basil ecotypes leaves after 4 h, and this UV-B effect was more severe after 48 h from the beginning of the experiment (during recovery) in almost sweet basil accessions tested, indicating that these are the most sensitive ecotypes in which such high energy light causes inhibition of the photosynthetic machinery and severe signs of UV-stress injuries (Figure 1). High UV-B radiation causes a reduction in photosynthesis, associated with decreased fluorescence, parameter such as Fv/Fm, indicating injury to the photosystems in several plants [25,27,40–42]. In contrast, the UV-treated OCI160 ecotype was clearly much more resistant to high UV-B stress and showed the highest Fv/Fm value at 48 h during recovery (Figure 2) and less signs of UV-stress injuries compared to others (Figure 1). In different Arabidopsis accessions grown under high UV-B dose, higher Fv/Fm values were interpreted as a greater protective capability, i.e., increased UV-B tolerance [41].

The performance of the photosynthetic apparatus plays a key role in the physiological status and vitality of plants under environmental stress.

Chlorophyll *a* fluorescence transient data were analyzed by using the JIP-test to quantify the physiological damage to photosynthetic machinery under high UV-B radiation in basil leaves [39,43,44]. In this study, the specific and phenomenological fluxes were significantly affected by UV-B radiation. Specific energy fluxes (TR_0/RC, ET_0/RC) of all ecotypes decreased under UV-B treatment as compared to not treated ones, while DI_0/RC and TR_0/RC of all ecotypes increased (Table 2). However, OCI160 plants showed a marginal alteration (decline and increase) in all these parameters. The higher average absorption (ABS_0/RC) and the greatest values of dissipation per reaction center (DI_0/RC) associated with concomitantly lower value of electron transport (ET_0/RC), trapping rate (TR_0/RC) per active PSII RC (ET_0/RC) and the decreased number of active PSII RCs per leaf cross section, RC/CS_0, observed in the other accessions (OCI: 142, 148, 149, 30, 102, 118, 126) might indicate increased UV-B stress sensitivity in these plants compared to OCI160. A large increase in the value of ABS/RC can be due to the inhibition of a fraction of PSII centers acting as permanently close centers [45,46], which leads to the disruption in the transport and capture of electrons by photosynthetic systems, thus a low photosynthetic performance in the sensitive ecotypes.

Similarly, the behavior of the phenomenological fluxes per cross section (ABS/CS_0, TR_0/CS_0, ET_0/CS_0, and DI_0/CS_0) under high UV-B radiation indicates a reduced overall processing of light energy with different degrees. It seems that the higher susceptibility of the majority sweet basil ecotypes (OCI: 142, 148, 149, 30, 102, 118, 126) to acute UV-B, may be related to a strong decrease in ET_0/CS_0 and TR_0/CS_0 (Figure 3B,C) due to the conversion of active reaction centers into inactive reaction centers, as indicated by lower RC/CS_0 values [47], thus reducing the efficiency of trapping and the PSII activity. Furthermore, the increase of DI_0/CS_0 represents a damage of electron transfer from the PSII to the photosynthetic machinery as shown by the significantly lower values of ET_0/CS_0 in sensitive ecotypes and subsequently the energy received must be released as heat (Figure 3D). Similar results have been reported in heath plants under drought stress [40], in quinoa leaves under drought stress [48], in ornamental plants under salt stress [34] in spinach leaves under low pH [49] and in tomato leaf under heat stress [50].

In contrast, the most tolerant ecotype (OCI160) showed a more efficient electron transport rate and a lower level in energy dissipation which might establish a consistent flow of electrons, improve the repair of photosystem II (PSII) and facilitate the ability of the donor and acceptor sides of PSII to deliver electrons. Thus, high UV-B dose resulted in a general decrease in photosynthetic efficiency by reducing flux ratios for Fv/Fm, TR_0/CS_0, ET_0/CS_0, and elevated flux ratios for DI_0/CS_0 and triggering perturbations within the photosynthetic apparatus that might exceed the PSII repair capacity of sensitive ecotypes. Taken together, these results suggest that physiological parameters, associated with the

leaf functionality of the photosynthetic machinery, may be used as reliable biomarkers to discriminate among ecotypes with a different tolerance to high UV-B light. Many investigations have reported that UV-B light stimulates the accumulation of phenolic compounds in the leaf epidermis to screen out UV damaging irradiation [25,27,51–54].

O. basilicum is considered a rich source of natural phenolic compounds [18,19,55] and their accumulation is strongly stimulated by UV-B light conditions [25,56–60]. In this study and in agreement with previous investigations [24,27], the main caffeic acid derivate detected at a significant level in sweet basil leaves was RA.

The accumulation of RA increased with the UV-B light treatment in OCI142, OCI148, OCI30, OCI160, and OCI102 ecotypes, with the highest concentration measured in OCI160 leaves (Figure 4). Levels of UV tolerance with respect to protection mechanisms differ considerably between genera, species, and even closely related cultivars [61]. The higher accumulation of RA in OCI160 leaves in comparison to the levels detected in other sweet basil ecotypes suggest an essential role of epidermal screening in UV protection, preventing chronic photoinhibition and promoting rapid photosynthetic recovery. The hydroxycinnamic acids are considered particularly effective in screening out UV-B radiation because they absorb effectively in the UV-B spectral region [62]. In addition, because RA has high radical-scavenging activity, its accumulation can counteract the increase in the intracellular level of ROS, preventing the damaging effect of UV-B radiation [63].

In conclusion, in *O. basilicum* leaves, synthesis of RA was strongly enhanced by high UV-B radiation in OCI160, showing fewer signs of UV-stress injuries compared to all other ecotypes. The other ecotypes did not increase the concentration of RA or synthesized significantly lower amounts of RA, compared to OCI160. Hence, this condition might diminish UV-B screening ability and, hence, increased susceptibility to UV-B damage, with a negative effect on photosynthetic performance. Our data indicate that UV-B-dependent inhibition of leaf functionality reported in the tolerant ecotype (OCI160), recovered in parallel to the increase of RA for UV-B radiation, suggesting that PSII is protected against damaging UV-B irradiation.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7050101/s1, Table S1.

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