

## Article

# Photosynthesis, Yield and Quality in Wild Rocket (*Diplotaxis tenuifolia* L.) under Photoluminescent Greenhouse Covers

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**Abstract:** Manipulation of light spectral composition is a useful tool to drive morphological, physiological and metabolic responses in several crops, ultimately improving yield and quality. Novel materials for greenhouse covering are being developed in order to make a better use of the available sunlight: among these are the cover films or panels incorporating fluorescent additives which are able to convert UV solar radiation into visible light. In this research, we compared the physiological traits and the agronomical performance of wild rocket grown in pots in the winter–spring season, under four different greenhouse prototypes covered with poly-methyl-methacrylate (PMMA)-based panels. PMMA panels doped at 3% (Dop3) or 7% (Dop7) *w/w* with a blend of rare-earth elements (partially converting the solar UV radiation to red and blue wavelengths) were compared with an undoped (UD) and a whitewashed (WW) PMMA greenhouse. The rocket yield was higher in Dop3 (+30%), while it was unaffected in Dop7 and lower in WW (−39%), compared to the control (6.06 kg m<sup>−2</sup>). The leaf greenness decreased while both the ABTS and the hydrophilic antioxidant activities increased under the doped and the whitewashed greenhouses. The Dop3 treatment provided the best results in terms of yield and quality of greenhouse wild rocket in winter–spring cycle. However, the analysis of OJIP kinetics of chlorophyll fluorescence revealed that the main factor affecting the photosynthetic performance was the light intensity inside each greenhouse rather than the modulation of light spectrum, because of the different shading properties of the doping and whitewashing treatments. Although these results did not allow us to distinguish between the combined effects of shading and light spectrum modulation, the use of photoluminescent covers can be foreseen as a promising innovation in greenhouse horticulture.

**Keywords:** poly-methyl methacrylate (PPMA); rare earths; chlorophyll fluorescence; OJIP; photochemistry; photosynthetic photon flux density (PPFD)



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## 1. Introduction

Compared to an open field, protected cultivation allows for off-season production of vegetables at a higher plant density, while optimizing the use efficiency of cultivation inputs and increasing the crop yield and product quality.

Light is a key factor for plant growth in greenhouse horticulture and the fine control of the light environment is still a challenge, since the natural light intensity is reduced to a varying extent, depending on the greenhouse design, on the optical properties of the cover material (glass or plastics), and by changes in these last features due to ageing, damages, and dust deposition [1]. The amount of light (intensity and duration) and its quality (spectral composition) affect the plant behaviour throughout the entire life cycle, as plants use light both as the energy source for photosynthesis and as a signal regulating many other fundamental processes of growth and development in photomorphogenesis [2]. On these bases, in the last decade, supplementary lighting with specific wavelengths has been employed to induce morphological, physiological, and metabolic responses in vegetable crops, by using light-emitting diodes (LEDs). Compared to traditional light sources, LEDs allow to tune the intensity and spectrum to meet the light requirements of each plant species. These can be modified according to the phenological phase of plants or in order to trigger specific responses, such as the synthesis of, e.g., antioxidants or other bioactive compounds which are known to benefit human health [3]. However, the use of LEDs at a commercial scale is still limited by several constraints, including the high cost for the hardware and energy power supply.

Therefore, the modulation of the sunlight spectrum by means of innovative greenhouse cover materials could be preferred to artificial lighting in terms of both economical and environmental sustainability. A range of cover materials for greenhouses are available, each one characterized by different optical properties which modify the light environment (light intensity, diffusion and spectrum) in turn affecting crop performance in different ways [4]. In recent years, new materials have been developed with the ability to modify the properties of the transmitted light [5]. For instance, photoselective films are able to filter the solar radiation, allowing or blocking the transmission of specific wavelengths while diffusive glasses or plastics (incorporating interference pigments, gas microbubbles, or hollow glass microspheres) are used for improving lighting uniformity inside the greenhouse [6]. Moreover, light conversion agents, from green (G) to red (R), ultraviolet (UV) to red (R), and UV to blue (B), can be used as dopants to modulate the proportion of B and R, and the red/far-red ratio (R/FR) [7]. By adjusting the light spectrum and intensity inside a greenhouse, it is possible to regulate the plant photosynthetic metabolism and photomorphogenic responses, ultimately controlling plant growth and product quality. More recently, the interest has been shifting toward a novel type of photoselective material, partially transmitting variable proportions of the UV radiation (particularly UV-B) which are known to affect the plant secondary metabolism, resulting in an accumulation of bioactive compounds (such as phenolics, carotenoids and glucosinolates). These studies support the idea that UV-B crossing plastic films in greenhouse crops may be used for the production of natural health-promoting food [8].

Light conversion agents are classified into fluorescent dyes and organic or inorganic rare-earth complexes [9]. Some agricultural films have been obtained by adding fluorescent dyes converting UV radiation into blue-violet or red-orange wavelengths [9]. Films doped with FR-absorbing dyes were as efficient as chemical growth regulators or  $\text{CuSO}_4$  filters in controlling the plant height in bell pepper and chrysanthemums [10]. Recent research focused on luminescent properties of some rare-earth-incorporating materials able to convert UV, visible (VIS) or infrared (IR) radiation into R and/or B wavelengths [11–13]. A highly efficient converter of UV, VIS and IR into R, implementing a luminescent material containing  $\text{P}_2\text{O}_5\text{-Li}_2\text{O-Al}_2\text{O}_3\text{-Sb}_2\text{O}_3\text{-MnO-Eu}_2\text{O}_3\text{-Er}_2\text{O}_3\text{-Yb}_2\text{O}_3$ , has been developed and successfully tested on phosphate glass [14]. A number of different cover materials, either glass- or organic polymer-based, doped with blends of rare-earth elements, have been developed and characterised for greenhouse covering [15].

In this research we evaluated the use of poly-methyl-methacrylate (PMMA) panels doped with a blend of rare-earth elements as greenhouse cover materials. PMMA doping resulted in a photoluminescent effect, converting the solar UV radiation into red and blue

wavelengths. Red light is effective in sustaining photosynthesis and it also controls some photomorphogenetic response (e.g., through changes of the R:FR ratio) [16].

The efficiency of photosynthesis can be measured non-destructively *in vivo* by using the method of chlorophyll *a* (Chl *a*) fluorescence analysis, which allows to detect the fine adjustments in the structure and in the functioning of the photosynthetic metabolism in response to environmental conditions [17,18]. Healthy plants, in the absence of major stress sources, use most of the light energy collected by the leaf pigments of the chloroplasts to power the photochemical phase of photosynthesis. However, a variable fraction of the absorbed energy may not be promptly channelled to the photochemical reactions and it is either dissipated as heat (thermal dissipation) or it is re-emitted as light by the chlorophyll pigments (chlorophyll fluorescence) [17]. At any time, there is an equilibrium among these pathways (photochemistry, thermal dissipation and fluorescence) which are in competition with each other for the use of light energy. Therefore, any increase in the efficiency of, e.g., photochemistry, will correspond either to decreased thermal dissipation or decreased fluorescence, or both. Chlorophyll fluorescence emission can be accurately measured with commercially available fluorimeters. Hence, although only 3–5% of the total absorbed light is dissipated through Chl *a* fluorescence, this fluorescence emission can be conveniently measured *in vivo* in the field using hand-held instruments, providing real-time fine details about the status of the photosynthetic machinery [19]. The analysis of chlorophyll fluorescence emission is based on the assumption that when a leaf is kept in the dark, its photosynthetic apparatus converts to a “dark-adapted” state (DAS), with all pigments of the light-harvesting complexes (or *antennae*) in their relaxed low-energy level, while the reaction centres (RCs) of the photosystems are in the “open” state, ready to receive excitation energy [20]. Upon illumination with a strong flash of light, within a very short time (<1000 ms), the excitation energy flowing from the *antennae* is transferred to the RCs which temporarily turn to an excited “closed” state until they can start the sequence of photochemical reactions of photosynthesis. As a consequence, all of the excess energy, collected by the *antennae* that cannot be transferred to the “closed” RCs, is dissipated as fluorescence. A fast increase in fluorescence emission occurs, from a minimum level ( $F_0$ ) to a maximum ( $F_m$ ) intensity, following a polyphasic induction kinetic, known as OJIP kinetics or fast transients of chlorophyll fluorescence [21,22]. The fluorescence signals, induced by strong illumination, are recorded with a high-time-resolution fluorimeter over a 2000 ms time course. From the measurement of the extremes  $F_0$  and  $F_m$ , the basic fluorescence parameters can be calculated:  $F_v/F_m$  (related to the maximum quantum yield of PSII in DAS) and  $F_v/F_0$  (proportional to the activity of the water-splitting complex) [23].

In previously published reports, we evaluated the use of innovative greenhouse cover materials, including diffusive films and photoluminescent poly-methyl-methacrylate (PMMA) panels doped with a blend of rare-earth elements, for the cultivation of vegetables such as wild rocket [24,25], lamb’s lettuce [26], spinach [27] and lettuce [28]. Based on our expertise, in this research, we studied the photosynthetic efficiency, yield and qualitative traits of wild rocket grown under greenhouse prototypes covered with PMMA doped at 3% (Dop3) or 7% (Dop7) *w/w* with a blend of rare-earth elements. PMMA doping, in addition to producing the photoluminescent effect, also resulted in a lower light transmittivity. Therefore, the two doped PMMA greenhouses were compared with a whitewashed PMMA greenhouse as a shaded control as well as with an undoped (UD) control greenhouse.

## 2. Materials and Methods

### 2.1. Experimental Design, Setting, and Crop Management

The experiment was carried out at the Department of Agricultural Science of the University of Naples Federico II (Portici, Naples, Italy), in the spring season of 2022. Plantlets of wild rocket (*Diplotaxis tenuifolia* L.) cv. ‘Reset’ (Maraldi Sementi Srl, Cesena, Italy) were transplanted on 1 February in pots (0.26 m diameter, 12 L capacity), at a plant density of 18 plants per  $m^2$ . Pots were filled with sandy soil (91.0% sand, 4.5% silt, 4.5%

clay), with pH 6.6, organic matter 2.6%, total N 1112 mg kg<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> 127.2 mg kg<sup>-1</sup>, and K<sub>2</sub>O 471.8 mg kg<sup>-1</sup>.

Pots were placed in small prototype greenhouses (length 100 cm, width 70 cm, height 70 cm), under four PMMA-based panels (described in Section 2.2):

- Undoped (UD);
- Doped with rare earths at 3% *w/w* (Dop3);
- Doped with rare earths at 7% *w/w* (Dop7);
- Whitewashed with paint (WW).

Six pots (replicates) were placed under each cover.

Nitrogen was applied at the dose of 18 kg ha<sup>-1</sup>, as ammonium nitrate (34%) several times for the first 18 days after the transplant and then about 7 days after each harvest. The water losses by evapotranspiration were estimated with the Hargreaves method and fully restored [28].

The harvests were performed in 4 cuts as follows: harvest I (17 March, at 44 days after transplant), harvest II (6 April, at 64 days after transplant), harvest III (26 April, at 84 days after transplant), and harvest IV (19 May, at 107 days after transplant).

## 2.2. Photoluminescent PMMA Panels for Greenhouse Covering

The poly-methyl methacrylate (PMMA) panels were produced by the cell-casting method: liquid MMA monomer was poured between two flat sheets of toughened glass, sealed with a rubber gasket and then heated for polymerization. Different proportions (3% or 7% *w/w*) of a rare-earth blend were added to PMMA to obtain the doped panels. The rare-earth blend consisted of two photoluminescent pigments converting red and blue wavelengths. The red component was due to CaS:Eu (Europium and Dysprosium), while the blue one was due to Sr<sub>4</sub>Ca<sub>4</sub>Al<sub>22</sub>O<sub>41</sub>:Eu, Dy<sup>+3</sup>, Nd<sup>+3</sup>, B<sub>3</sub> (calcium oxide, Strontium oxide, Aluminium Oxide and Europium Oxide), at the weight ratio of 70/30. The resulting sheets were transparent in the case of undoped PMMA, and white opalescent in the case of doped PMMA (Picture 1).



**Picture 1.** Overview of the greenhouse prototypes used in the experiment. From left to right: PMMA doped with rare earths at 3% (Dop3), doped with rare earths at 7% (Dop7), undoped (UD) and whitewashed (WW).

## 2.3. Light Spectra, Total Irradiance, PAR, and Temperature Measurements

The light spectra inside and outside the greenhouses were measured with an Optics Maya 2000 Pro spectrophotometer (Ocean Insight, Oxford, UK; spectral range 165–1100 nm), set at a 14 ms integration time. The total irradiance in the spectral range 400–1050 nm was measured using an HD2102.2 photoradiometer (Delta Ohm, Padua, Italy), equipped with a LP471RAD (Delta Ohm) radiometric probe with a cosine corrector. Light intensity, or photosynthetic photon flux density (PPFD) in the 400 to 700 nm PAR (photosynthetically active radiation) range, was measured in  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  with the PAR sensor of a PSI PAR-FluorPen FP 110/D (Photon Systems Instruments, Drásov, Czech Republic) on the same day of the chlorophyll fluorescence measurements.

Air temperature was monitored during the whole growing period with a Vantage Pro2 weather station (Davis Instruments, Hayward, CA, USA), at an hourly interval.

#### 2.4. Plant Growth and Yield

Plant growth was evaluated at each harvest in terms of number of leaves per pot, average leaf weight, and total fresh weight. Marketable yield was expressed in  $\text{kg m}^{-2}$ . Dry weight and dry matter percentage were calculated on leaf sub-samples which were oven-dried at  $70\text{ }^{\circ}\text{C}$  until constant weight was reached.

#### 2.5. Chroma Meter Measurements and Pigments Determinations

The colour characteristics were measured in rocket leaves at each harvest, using a CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan), in the middle portion of the adaxial surface (between the midrib and the margin) of fully expanded leaves, in 5 leaves per pot (30 leaves per treatment). Colour measurements were expressed through the CIELAB (Commission international de l'éclairage) parameters  $L^*$  (brightness),  $a^*$  (redness component), and  $b^*$  (yellowness component).

The content of chlorophyll pigments was determined indirectly as leaf greenness, in SPAD (Soil Plant Analysis Development) units, by using a portable Konica Minolta chlorophyll meter (model SPAD-502, Tokyo, Japan). Measurements were carried out on 8 leaves per pot (48 leaves per treatment), immediately prior to each harvest.

Chlorophyll *a*, chlorophyll *b* and carotenoids were determined according to Lichtenthaler and Buschmann [29]. Briefly, frozen leaf samples were extracted in pure acetone and, after centrifugation at  $3000 \times g$  for 5 min, the absorbance of the supernatant was measured at 662, 645, and 470 nm, with a spectrophotometer Hach DR 2000 (Hach Co., Loveland, CO, USA). Total chlorophylls were calculated as the sum of chlorophyll *a* and *b*.

#### 2.6. Antioxidant Activity, Phenols and Total Ascorbic Acid Content

ABTS antioxidant activity (ABTS AA) was measured on 200 mg freeze-dried leaves extracted with distilled water as described by Re et al. [30] and it was expressed as  $\text{mmol of Trolox } 100\text{ g}^{-1}\text{ dw}$ . Hydrophilic antioxidant activity (HAA) was assayed on 200 mg freeze-dried leaves extracted in methanolic extract according to Fogliano et al. [31] and it was expressed as  $\text{mmol ascorbic acid } 100\text{ g}^{-1}\text{ dw}$ . Total phenols were determined in methanolic extracts as previously described [25], using the Folin–Ciocalteu method [32] with gallic acid as a standard. Total ascorbic acid (TAA) was spectrophotometrically assayed according to Kampfenkel et al. [33] and it was expressed as  $\text{mg ascorbic acid } \text{g}^{-1}\text{ fw}$  on 100 g fw.

#### 2.7. Chlorophyll Fluorescence Measurements

Chlorophyll *a* fluorescence kinetics were recorded in the field on 10 March 2022 prior to Harvest I, on randomly sampled fully expanded leaves, using a PAR-FluorPen FP 110/D portable fluorimeter (Photon Systems Instruments, Drásov, Czech Republic) equipped with detachable leaf clips. Prior to measurements, the leaves were dark-adapted for 30 min using the fluorimeter leaf-clips, then Chl *a* fluorescence was induced by the fluorimeter internal LED blue light (455 nm), producing a saturating light pulse of  $2700\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$  and the fast rise of chlorophyll fluorescence was recorded for 2000 ms using the fluorimeter OJIP protocol, as described by Strasser et al. [17]. Each measurement was replicated ten times, between 10:30–11:30 (Central European Time).

Fluorescence data were processed using the FluorPen software ver. 1.1 (Photon Systems Instruments, Drásov, Czech Republic) and they were further analyzed using MS Excel 365.

#### 2.8. Statistical Analysis

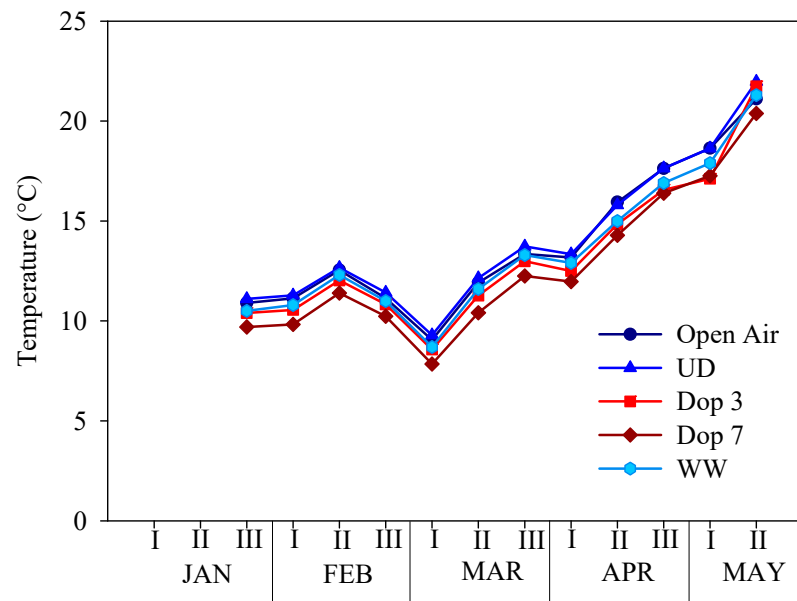
All data were subjected to 2-way analysis of variance (ANOVA) with the SPSS software package (SPSS version 22, Chicago, IL, USA). The means were separated using Tukey's Test at  $p \leq 0.05$ .



### 3. Results

#### 3.1. Air Temperature

Figure 1 shows the average daily temperatures recorded inside the different greenhouse prototypes and in the open air. In all the treatments, the temperature reached the minimum value in the first week of March and the maximum in mid-May. Specifically, the outdoor temperature ranged between 9.0 °C to 21.1 °C, respectively. The undoped PMMA determined a general increase in temperature (+2 °C on the average of the 12 weeks), while doping treatments with rare earths and whitewashing decreased the thermal level compared to outside (−0.6, −1.2, and −0.4 °C, for Dop3, Dop7, and WW, respectively).



**Figure 1.** Average temperature recorded in open air and in the greenhouse prototypes under PMMA cover panels: undoped (UD), doped with rare earths at 3% (Dop3) and 7% (Dop7), and whitewashed. Roman numerals I to III indicate the decades of each month.

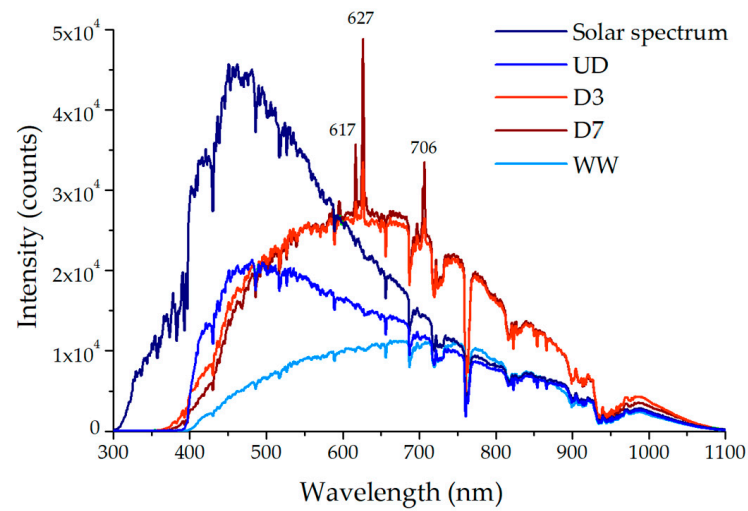
#### 3.2. Optical Characterization of PMMA Panels

##### 3.2.1. Transfer of Light Spectrum

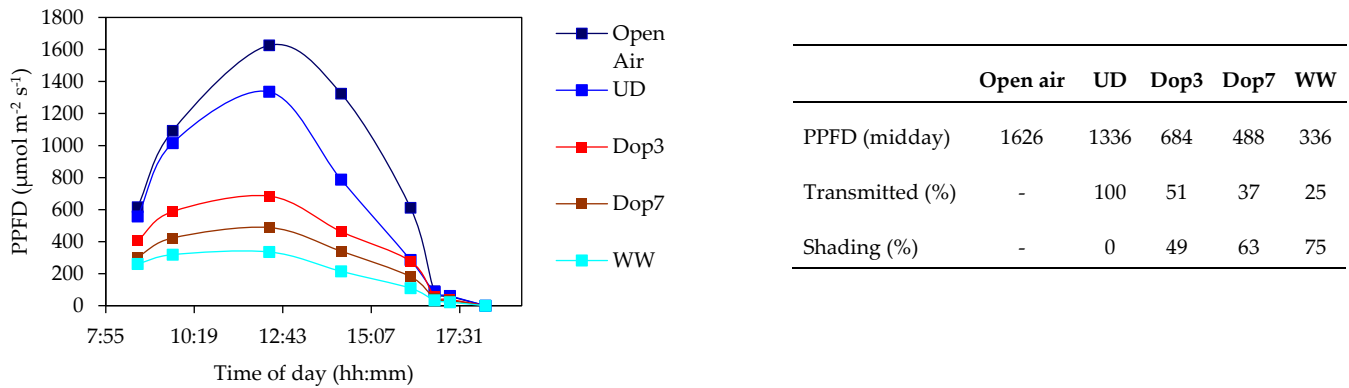
The optical properties of undoped and doped PMMA panels were investigated through two different UV-Vis spectrophotometers used for spectral measurements both in laboratory and in the field. Figure 2 shows the incident solar radiation (solar spectrum) and the transmission spectra recorded under undoped (UD), doped with 3% (D3) and 7% (D7) of rare earths and whitewashed (WW) PMMA panels. The UD PMMA control panel totally absorbed the incident UV radiation without emitting supplementary wavelengths. Conversely, under the doped panels, the absorbed UV radiation was converted into the emission of three different peaks in the red region because of the photoluminescent effect. The panel with the highest doping proportion (D7) exhibits more pronounced photoluminescence peaks.

##### 3.2.2. Transfer of Light Intensity

The photoluminescent greenhouse covers also differed in their overall light-shading power. Figure 3 shows the daily course of Photosynthetic Photon Flux Density (PPFD) measured at the plant level prior to Harvest I (10 March 2022). The peak of light intensity at midday was substantially shaded in greenhouse by the undoped PMMA cover (UD) compared to the outside. The doping and whitewashing treatments differed in their shading power. Specifically, inside the D3, D7 and WW greenhouses, the PPFD was, respectively, 49%, 63% or 75% lower compared to the UD control (Figure 3).



**Figure 2.** Spectra of unfiltered solar light compared with light transmitted through the different greenhouse cover panels.



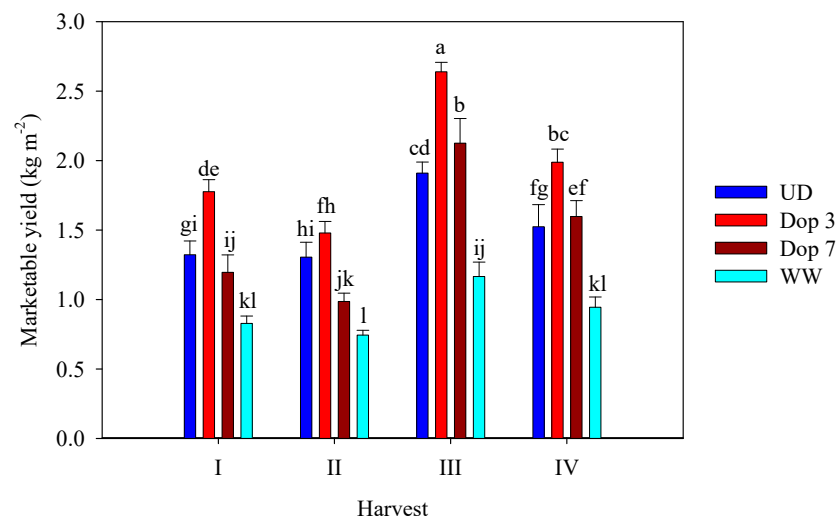
**Figure 3.** Light intensity and shading power (% relative to UD) recorded under the different greenhouse covers during the day of the harvest I (10 March).

### 3.3. Plant Growth and Yield

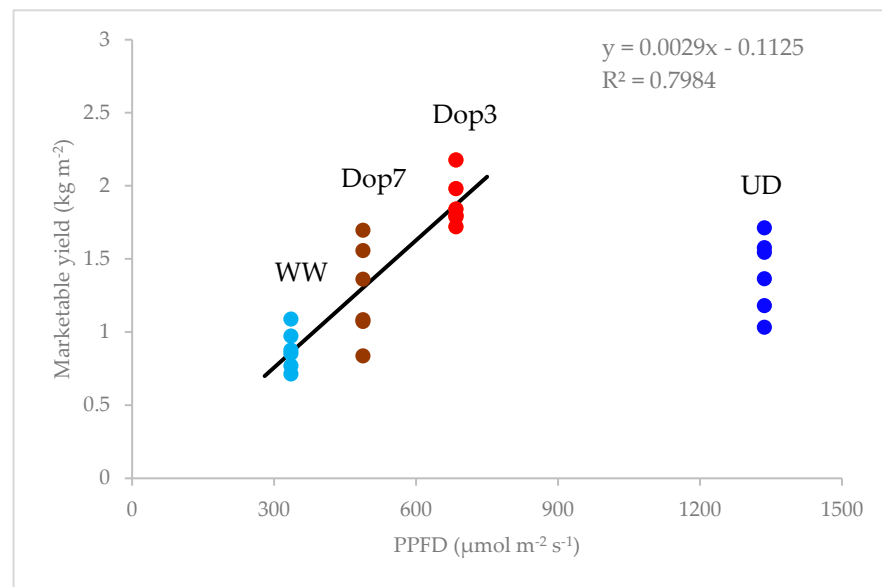
The harvests were performed on 17 March (I), 6 April (II), 26 April (III), and 19 May (IV). In control plants grown under undoped PMMA panels, the yield of rocket ranged between the minimum of  $1.32 \text{ kg m}^{-2}$  (average of the harvests I and II) and the maximum of  $1.91 \text{ kg m}^{-2}$  (harvest III) (Figure 4). Both the doping with rare earths and the whitewashing of PMMA influenced the crop productivity, with different effects among the covering treatments and the harvest time (Figure 4). Specifically, doping at 3% (Dop3) significantly increased the leaf production at all the harvests except the second one, while doping at 7% (Dop7) did not affect the plant productivity at the first and the last cut, while reducing and increasing the yield at the second and the third ones, respectively, compared to the undoped cover. Whitewashing of PMMA always impaired the crop productivity compared to the control (Figure 4).

At the end of the whole growing period, the total yield varied from the lowest value of  $3.68 \text{ kg m}^{-2}$  under the shaded greenhouse (WW), to the maximum of  $7.88 \text{ kg m}^{-2}$  under PMMA doped with rare earths at 3% and was similar in the unshaded control and the panel doped at 7% ( $6.06 \text{ kg m}^{-2}$  in UD and  $5.91 \text{ kg m}^{-2}$  in Dop7).

A direct relationship between the marketable yields and the transmitted light intensity was recorded under the doped and whitewashed covers (Figure 5).



**Figure 4.** Marketable yield (in  $\text{kg m}^{-2}$ ) of greenhouse wild rocket as affected by the interaction between cover panels and harvest time (I: 17 March; II: 6 April; III: 26 April; IV: 19 May). Values are means  $\pm$  standard error. Different letters indicate significant differences according to Tukey's HSD test at  $p \leq 0.05$ .



**Figure 5.** Relationship between yield ( $\text{kg fresh weight m}^{-2}$ ) of wild rocket and PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) under the different greenhouse covers.

From a physiological point of view, the plant productive performance results from the combination of light intensity, light spectrum and temperature inside the greenhouses. In this respect, by plotting the marketable yield vs. the daily maximum value of PPFD, a significant ( $R^2 = 0.80$ ) positive linear correlation emerged in all the three light-screened greenhouses (WW, Dop7 and Dop3), while a lower productivity at the highest PPFD level resulted in the UD greenhouse (Figure 5). Table 1 shows the interaction between the cover treatments and the harvest time on yield parameters in rocket plants along the four harvests. In general, under all the covering types, the number of leaves was higher at harvest I, decreased at II and III, then raised again at harvest IV. Conversely, the leaf average fresh weight increased from the I to the III, then decreased in the IV (Table 1).



**Table 1.** Yield parameters in wild rocket as affected by the interaction between greenhouse cover and harvest time. Values are means  $\pm$  standard error, within each column different letters indicate statistically significant differences according to Tukey's HSD test: ns not significant; \* significant at  $p \leq 0.05$ ; \*\* significant at  $p \leq 0.01$ .

		Leaves		Dry Matter (%)
		Number of Leaves $m^{-2}$	Mean Leaf Weight (g)	
UD	I	4309.3 $\pm$ 646.9 cd	0.168 $\pm$ 0.019 gi	12.7 $\pm$ 0.7
	II	3633.3 $\pm$ 541.1 e	0.192 $\pm$ 0.013 fh	11.0 $\pm$ 0.4
	III	3903.8 $\pm$ 522.4 de	0.264 $\pm$ 0.015 cd	12.7 $\pm$ 0.5
	IV	5347.7 $\pm$ 1007.4 b	0.156 $\pm$ 0.017 i	13.2 $\pm$ 0.8
Dop3	I	4885.3 $\pm$ 1157.3 bc	0.211 $\pm$ 0.026 ef	12.5 $\pm$ 0.2
	II	3348.6 $\pm$ 344.2 ef	0.235 $\pm$ 0.011 de	10.4 $\pm$ 0.4
	III	3783.4 $\pm$ 506.3 de	0.379 $\pm$ 0.027 a	10.8 $\pm$ 0.5
	IV	6483.9 $\pm$ 750.0 a	0.164 $\pm$ 0.007 hi	12.6 $\pm$ 0.4
Dop7	I	3682.2 $\pm$ 440.0 de	0.168 $\pm$ 0.012 gi	11.7 $\pm$ 0.3
	II	2663.3 $\pm$ 157.2 gh	0.197 $\pm$ 0.012 fg	10.1 $\pm$ 0.4
	III	3410.7 $\pm$ 556.2 ef	0.339 $\pm$ 0.035 b	11.1 $\pm$ 0.3
	IV	4547.4 $\pm$ 939.4 c	0.185 $\pm$ 0.013 fi	11.9 $\pm$ 0.8
WW	I	2593.6 $\pm$ 221.9 gh	0.172 $\pm$ 0.008 gi	10.7 $\pm$ 0.1
	II	2078.5 $\pm$ 119.1 h	0.191 $\pm$ 0.006 fh	10.8 $\pm$ 0.8
	III	2221.3 $\pm$ 106.5 h	0.279 $\pm$ 0.021 c	10.5 $\pm$ 0.6
	IV	2847.3 $\pm$ 231.3 fg	0.179 $\pm$ 0.015 gi	10.8 $\pm$ 0.3
Significance				
Doping (D)		**	**	**
Harvest (H)		**	**	**
Interaction D $\times$ H		*	*	ns

Regarding the average of the harvest periods, the total number of leaves reached the highest value in plants under the clear PMMA and the cover doped at 3%, while it was reduced by doping at 7% and whitewashing. Averaged on the four cuts, greenhouse covers' leaf average fresh weight was 0.217 g leaf<sup>-1</sup> and the dry matter percentage was 11.47%.

### 3.4. Leaf Colour Parameters and Spad Index

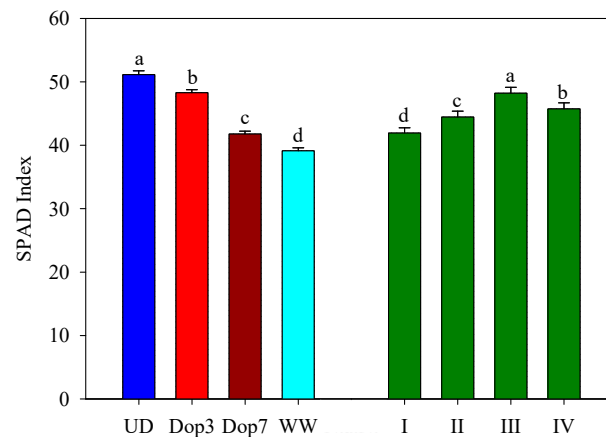
The CIELAB colour parameters recorded in rocket leaves at each harvest are reported in Table 2. The PMMA doping with rare earths did not influence the leaf brightness, while whitewashing (WW) significantly reduced the L value respect to all the other covers (Table 2). Compared to Control, both the redness (a\*) and the yellowness (b\*) components were unaffected by doping, while they were reduced by whitewashing (Table 2).

**Table 2.** Colour parameters (brightness L\*, redness a\*, yellowness b\*) in leaves of greenhouse-grown wild rocket as affected by greenhouse cover and by harvest time. Values are means  $\pm$  standard error, within each column different letters indicate statistically significant differences according to Tukey's HSD test: ns not significant; \* significant at  $p \leq 0.05$ ; \*\* significant at  $p \leq 0.01$ .

	L*	a*	b*
Cover treatment			
UD	40.68 $\pm$ 0.42 ab	-12.38 $\pm$ 0.23 bc	18.40 $\pm$ 0.42 ab
Dop3	41.29 $\pm$ 0.34 a	-11.81 $\pm$ 0.25 ab	18.21 $\pm$ 0.45 ab
Dop7	41.11 $\pm$ 0.36 ab	-13.04 $\pm$ 0.26 c	19.17 $\pm$ 0.54 a
WW	40.42 $\pm$ 0.36 b	-11.62 $\pm$ 0.25 a	17.23 $\pm$ 0.47 b
Harvest			
I	38.84 $\pm$ 0.25 c	-11.10 $\pm$ 0.24 a	16.30 $\pm$ 0.43 b
II	41.03 $\pm$ 0.23 b	-12.15 $\pm$ 0.21 b	17.32 $\pm$ 0.31 b
III	41.44 $\pm$ 0.31 ab	-13.23 $\pm$ 0.27 c	19.72 $\pm$ 0.48 a
IV	42.20 $\pm$ 0.29 a	-12.36 $\pm$ 0.14 b	19.67 $\pm$ 0.26 a
Significance			
Doping (D)	**	**	**
Harvest (H)	*	**	**
Interaction D $\times$ H	ns	ns	ns

The parameters  $L^*$  and  $b^*$  increased from the first to the last two harvests, while the component  $a^*$  reached the highest value at the third harvest and then decreased at the last one (Table 2).

Figure 6 shows the average effects of the harvest time and the greenhouse cover on the green colour intensity of rocket leaves, expressed as SPAD index. The leaf greenness reached the highest value under the undoped PMMA, while it was reduced by both the doping treatments, with decreasing values from Dop3 to Dop7, and the whitewashing. The SPAD index increased from the first to the third harvest, then it decreased in the last one.



**Figure 6.** SPAD index (leaf chlorophyll content) in wild rocket as affected by greenhouse cover and harvest time. Values are means  $\pm$  standard error, different letters indicate statistically significant differences according to Tukey's HSD test at  $p \leq 0.05$ .

### 3.5. Antioxidant Activities, Total Phenols, and Total Ascorbic Acid

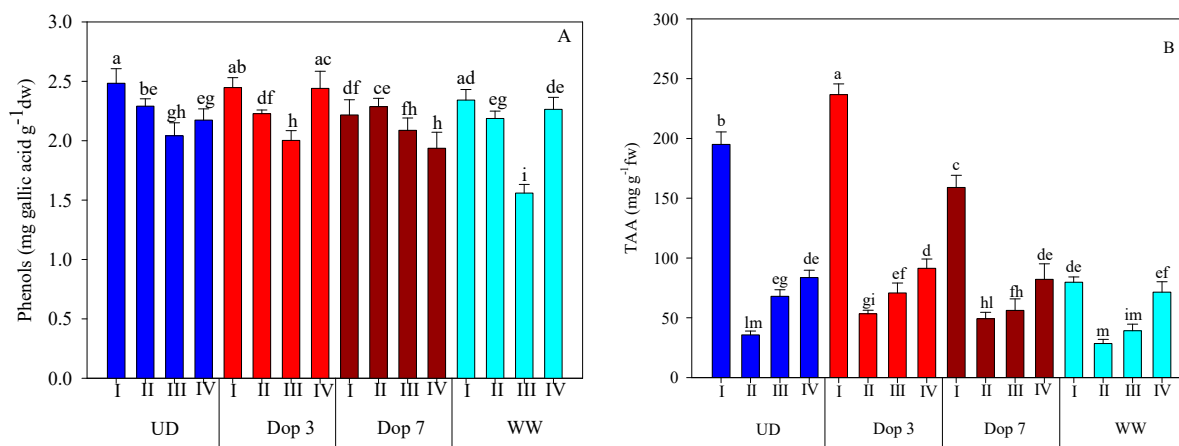
ABTS antioxidant activity (ABTS) and hydrophilic antioxidant activity (HAA) in greenhouse wild rocket leaves were influenced by both the cover type and the harvest time (Table 3). HAA increased under both the doping rates and the whitewashing compared to the clear PPMA.

**Table 3.** Antioxidant activity (ABTS) and hydrophilic antioxidant activity (HAA) in leaves of wild rocket as affected by greenhouse cover and by harvest time. Values are means  $\pm$  standard error, within each column different letters indicate statistically significant differences according to Tukey's HSD test: ns not significant; \*\* significant at  $p \leq 0.01$ , \*\*\* significant at  $p \leq 0.001$ .

	ABTS	HAA
	mM Trolox 100 g <sup>-1</sup> dw	mM AA 100 g <sup>-1</sup> dw
Cover treatment		
UD	8.31 $\pm$ 0.24 b	13.54 $\pm$ 0.86 b
Dop3	8.40 $\pm$ 0.26 ab	15.88 $\pm$ 0.91 a
Dop7	8.54 $\pm$ 0.25 ab	17.02 $\pm$ 0.96 a
WW	9.21 $\pm$ 0.27 a	19.05 $\pm$ 1.06 a
Harvest		
I	8.13 $\pm$ 0.13 b	10.62 $\pm$ 0.37 c
II	8.25 $\pm$ 0.20 b	18.57 $\pm$ 0.93 ab
III	8.32 $\pm$ 0.18 b	19.70 $\pm$ 0.90 a
IV	9.57 $\pm$ 0.37 a	16.60 $\pm$ 0.61 b
Significance		
Doping (D)	**	***
Harvest (H)	***	***
Interaction D $\times$ H	ns	ns

The ABTS showed similar values in the first three harvests (from March to April) and increased in the fourth one (May), while the HAA ranged from the minimum value in March to the maximum at the end of April, then decreased in May (Table 3).

A significant interaction between the greenhouse cover and the harvest time was found in the leaf concentration of phenols and total ascorbic acid. In general, the content of phenols decreased from the first to the third then increased in the fourth harvest (Figure 7). The values recorded under the clear and the doped PMMA were similar among the treatments in the first three harvests, while they were higher in Dop3 and lower in Dop7 compared to control in the last one. The whitewashed cover determined the lowest phenol content, recorded in harvest III (Figure 7).



**Figure 7.** Total phenols (A) and total ascorbic acid (TAA) (B) in leaves of wild rocket as affected by the interaction between greenhouse cover and harvest time. Values are means  $\pm$  standard error, different letters indicate statistically significant differences according to Tukey's HSD test at  $p \leq 0.05$ .

The total ascorbic acid (TAA) showed the highest value in the product harvested in March, then decreased drastically and was always lower in the other harvests under all the cover types except WW, in which it raised to the initial value at the last harvest (Figure 7). This cover determined the lowest TAA value compared to the other treatments.

### 3.6. Leaf Chlorophyll (Chl) and Carotenoids Content

The doping treatment with rare earths at 7% determined a higher leaf Chl *a* content, while the whitewashing did not influence Chl *a* and reduced the carotenoids compared to the clear PMMA; neither the doping nor the whitening affected the total chlorophylls (Table 4).

In terms of influence of the harvest time, the leaf total chlorophyll and carotenoid concentration showed the highest values in the product of the first harvest, but were decreased in the second and the fourth harvest for chlorophyll, and in all the other harvests for carotenoids (Table 4).

### 3.7. Chlorophyll Fluorescence Analysis

The impact of the greenhouse cover materials on plant photochemistry was analyzed in the field on intact leaves by means of Chl *a* fluorescence measurement prior to harvest I and the JIP-test procedure was followed for analysis of the OJIP kinetics.

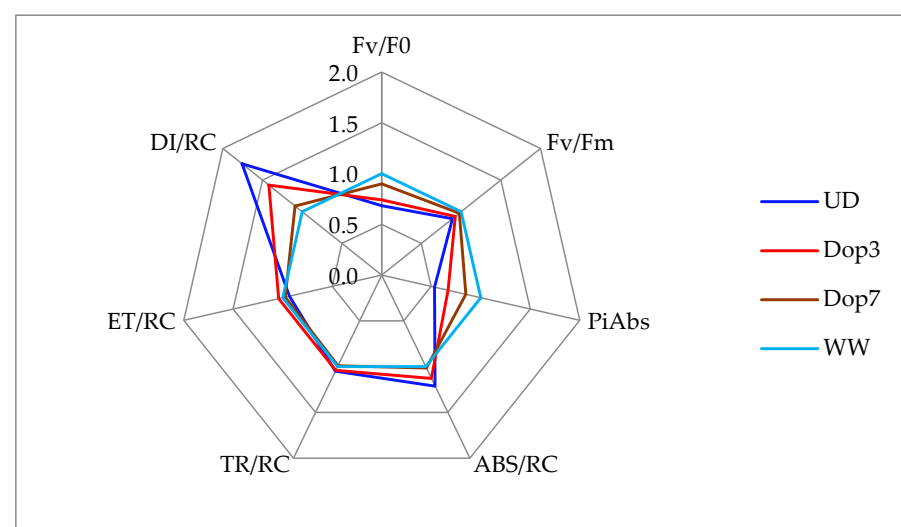
The calculated values of a selected subset of JIP-test parameters are reported in Table 5, while the relative variations of each parameter (normalized to the respective value measured under the WW greenhouse) are shown in the radar plot in Figure 8. The highest  $F_v/F_m$  and  $F_v/F_0$  ratios were recorded under the WW greenhouse, and progressively lower values under the Dop7 (not significantly different), Dop3 and UD greenhouses. The same trend was observed for the Performance Index ( $Pi_{Abs}$ ), which was 87% higher in WW compared to UD plants (Table 5 and Figure 8).

**Table 4.** Chlorophylls *a*, *b*, total chlorophyll and carotenoids in leaves of wild rocket as affected by greenhouse cover and by harvest time. Values are means  $\pm$  standard error, within each column different letters indicate significant differences according to Tukey's HSD test: ns (not significant); \* (significant at  $p \leq 0.05$ ), \*\* (significant at  $p \leq 0.01$ ); \*\*\* (significant at  $p \leq 0.001$ ).

Treatment	Chlorophyll <i>a</i> mg g <sup>-1</sup> fw	Chlorophyll <i>b</i> mg g <sup>-1</sup> fw	Total Chlorophylls mg g <sup>-1</sup> fw	Carotenoids $\mu$ g g <sup>-1</sup> fw
Cover treatment				
UD	0.846 $\pm$ 0.050 b	0.431 $\pm$ 0.020	1.355 $\pm$ 0.036	0.344 $\pm$ 0.004 ab
Dop3	0.932 $\pm$ 0.011 ab	0.451 $\pm$ 0.012	1.383 $\pm$ 0.022	0.352 $\pm$ 0.004 a
Dop7	0.959 $\pm$ 0.017 a	0.457 $\pm$ 0.017	1.415 $\pm$ 0.029	0.350 $\pm$ 0.004 a
WW	0.916 $\pm$ 0.017 ab	0.455 $\pm$ 0.019	1.371 $\pm$ 0.035	0.334 $\pm$ 0.006 b
Harvest				
I	0.978 $\pm$ 0.012 a	0.503 $\pm$ 0.016 a	1.482 $\pm$ 0.028 a	0.365 $\pm$ 0.005 a
II	0.900 $\pm$ 0.016 ab	0.439 $\pm$ 0.016 b	1.339 $\pm$ 0.025 b	0.346 $\pm$ 0.003 b
III	0.870 $\pm$ 0.052 b	0.431 $\pm$ 0.018 b	1.379 $\pm$ 0.032 ab	0.335 $\pm$ 0.005 b
IV	0.905 $\pm$ 0.014 ab	0.420 $\pm$ 0.014 b	1.325 $\pm$ 0.028 b	0.334 $\pm$ 0.004 b
Significance				
Doping (D)	*	ns	ns	**
Harvest (H)	**	*	**	***
Interaction D $\times$ H	ns	ns	ns	ns

**Table 5.** JIP-test parameters in leaves of greenhouse wild rocket at harvest I as affected by greenhouse covers. Within each line, different letters indicate significant differences among treatments according to Fisher's LSD test at  $p \leq 0.05$ .

JIP-Test Parameter	UD	Dop3	Dop7	WW
Fv/Fo	2.359 b	2.558 b	3.101 a	3.450 a
Fv/Fm	0.688 c	0.716 bc	0.755 ab	0.773 a
Pi <sub>Abs</sub>	0.708 c	0.889 bc	1.127 ab	1.327 a
ABS/RC	3.571 a	3.322 ab	2.987 bc	2.945 bc
TR/RC	2.387	2.365	2.253	2.273
ET/RC	1.111	1.242	1.164	1.194
DI/RC	1.184 a	0.957 ab	0.734 bc	0.673 bc



**Figure 8.** Chlorophyll fluorescence parameters in leaves of wild rocket at harvest I as affected by greenhouse covers. Quantum yields, Performance Index and Energy cascade parameters calculated according to the JIP-test procedure (see main text).

The energy cascade parameters (ABS/RC, TR/RC, ET/RC, DI/RC), describing the partitioning of the Absorbed light energy (ABS) into different energy fluxes which power the photochemical reactions in the chloroplasts, are shown in Table 5 (absolute values) and in Figure 8 (normalized values). Although only in the case of plants grown inside the UD greenhouse the ABS/RC statistically differed from Dop7 and WW, this parameter appears to follow a gradual increase from WW to UD, directly related to the light intensity available inside these greenhouses. Neither the trapping (TR) or the electron transport (ET) fluxes were affected by the different greenhouse light environments, as no significant difference was found among treatments. Contrastingly, the dissipation of absorbed energy (DI) was significantly higher under the UD greenhouse following a similar trend to the ABS flux.

#### 4. Discussion

The development of smart materials able to modify light intensity, diffusion, or spectral composition in protected cultivation is a promising research field and a mandatory step to increase the use efficiency of natural light and the crop productivity, while improving the sustainability of the greenhouse industry [5]. Among the light conversion agents, including inorganic and organic rare earths and fluorescent dyes, the former is cheaper, easy to be prepared and stored, and resistant to oxidation and high temperature; hence, it is suitable for the doping of greenhouse cover materials. In addition, some of them exhibit a high luminous efficiency, a wide conversion range of light wavelengths, and a spectrum emission matching the plant absorption [9].

In wild rocket grown in the winter–spring period in a Mediterranean climate, in small greenhouse prototypes covered with PMMA, we compared two doping concentrations, 3% and 7% in weight, with rare earths with photoluminescent pigments emitting red and blue light, to an untreated and a whitewashed cover as control for the shading effect of doping. In our experiment, the yield of rocket cv. ‘Reset’ grown under undoped PMMA in a 4-month cycle from February to May in the South of Italy reached  $6.06 \text{ kg m}^{-2}$  (Figure 4) and it was similar to those obtained with the same cultivar grown in the autumn–spring period under polyethylene thermal film in the same environment [25]. Doping PMMA panels with rare earths at 3% increased the total marketable yield by 30% compared to the untreated control (Figure 4), by raising both the number of leaves and the leaf average fresh weight (Table 1), at all the harvests except the second one (6 April). Conversely, doping at 7% did not affect the final plant productivity, since it did not change the biomass accumulation in the harvests of March and May, while it reduced and increased the yield at the first and second harvests of April, respectively, compared to the undoped control (Figure 4). The biomass productivity of a crop results from the interaction of multiple factors, including genetic potential (species and variety), environmental factors (such as nutrients and water availability), light spectrum, duration and intensity, and temperature, as well as biotic and abiotic stresses occurring during the growing cycle [34]. In our experiment, productive results (Figure 5) seemed to be also related to the intensity of light (Figure 3) in addition to the light spectrum (Figure 2) and air temperature (Figure 1) under the different greenhouse covers. Consistently, the greenhouse whitewashing always impaired the rocket yield, lowering the final yield by 39% compared to the clear PMMA (Figure 4), through a drastic reduction of the number of leaves (Table 1), hence a combined effect of a lower light intensity and a reduced photosynthetically active leaf surface, both limiting the plant assimilation rate. The lower productivity at the highest PPFD level inside the UD greenhouse (Figure 5) could be ascribed to the activation of photoprotection/photoinhibition processes, which physiologically protect the integrity and functionality of the photosynthetic apparatus at the cost of restricting, as a side effect, the assimilation of carbon dioxide [35].

In our previous experiment on lettuce grown in the fall–spring cycle in the same facilities, the PMMA doping with rare-earth blend at 5% (*w/w*) elicited a 36% increase in the marketable yield compared to the undoped cover [36]. Similarly, other studies reported higher fresh and dry weight under films converting green light to red light on several horticultural crops (lettuce, spinach, celery, and sweet potato) [37]. In lettuce, tomato, and



melon, among three different spectrum conversion greenhouse films, in R, B, and R + B, the R + B one increased the number of leaves and fresh weight of lettuce [38]. Similarly, in Chinese flowering cabbage under a film converting blue-violet to red-orange light, both the fresh and dry weight increased [39].

In general, doping treatments of PMMA determined a reduction of light transmittance, resulting in lower light intensity (Figure 3) and daytime temperature (Figure 1) compared to the undoped control. In our experiment, considering the solar irradiance of the cultivation period (increasing from February to May), this shading effect was probably negligible and did not impair the plant growth at the doping rate of 3%, making significant the positive effect of the enrichment in red wavelengths on the plant performance. Conversely, the higher shading rate of 7% doping was limiting for plant assimilation, also making ineffective the red augmentation, ultimately reducing the plant productivity. In this respect, it is reasonable to predict that the same doping treatments could exert different output depending on the specific light requirements of different crops as well as on different growing seasons. For instance, potentially better effects could be expected under a higher doping concentration in summer, with a greater conversion rate of UV in red light combined with higher shading (also reducing temperature, hence improving the microclimate in greenhouse), compared to lower doping.

As regards the photosynthetic metabolism (Table 5, Figure 8), the basic parameters derived from the Chl *a* fluorescence kinetics are the  $F_v/F_m$  and the  $F_v/F_0$  ratios. The  $F_v/F_m$  is a measure of the maximum quantum yield for primary photochemistry (or the conversion efficiency of absorbed light into photochemical reactions) while the  $F_v/F_0$  ratio is assumed to be proportional to the activity of the water-splitting and Oxygen Evolving Complex of the PSII and it has a wider sensitivity scale than  $F_v/F_m$  [23,40]. In our experiment, both  $F_v/F_m$  and  $F_v/F_0$  were the highest under the WW greenhouse, and progressively lower in Dop7, Dop3 and UD. This trend indicated that the efficiency of light conversion into photochemical reactions at the level of PSII Reaction Centers (RCs) was inversely related to the PPFD level inside the greenhouse. This result can be explained by the gradual initiation of photoinhibition processes in rocket leaves, involving the activation of photoprotective non-photochemical chlorophyll fluorescence quenching [41]. A similar tendency was recorded for the Performance Index ( $Pi_{Abs}$ ), which was 87% higher in WW compared to UD plants. The  $Pi_{Abs}$  is a very sensitive parameter combining different biophysical indices derived from the fluorescence transients. Overall,  $Pi_{Abs}$  is a measure of energy conservation from the absorption of photons by PSII until the reduction of intersystem (PSII-PSI) electron acceptors and it is a very useful tool for the evaluation of the photosynthetic performance of plants [22]. Similarly, Kalaji et al. [42] reported that  $Pi_{Abs}$  and other Chl *a* fluorescence parameters related to energy flux within PSII which were specifically affected under low or high light stress. The fluorescence parameters  $F_v/F_m$ ,  $F_v/F_0$  and  $Pi_{Abs}$  consistently confirm the inverse relationship between the efficiency of photochemistry and the PPFD intensity inside the greenhouses.

The “energy cascade” parameters (ABS/RC, TR/RC, ET/RC, DI/RC) are derived from the Theory of Energy Fluxes (TEF) [22,43,44] and they allow for a detailed analysis of the energy fluxes from the primary photochemical reduction of  $Q_A$  by PSII until the reduction of electron acceptors after PSI. ABS/RC is a measure of the light energy absorbed by PSII *antennae* and it is proportional to the total amount of chlorophyll per reaction center (RC), also providing a measure of the apparent *antenna* size. The gradual increase in ABS/RC from the WW- to UD-grown plants corresponds to the increasing PPFD levels inside the greenhouses. This result therefore matches the indirect measures of chlorophyll (SPAD indices) and it confirms that chlorophyll content in rocket leaves is positively related to the environmental light level. The sequence of increasing PPFD level under the different greenhouse covers (WW, Dop7, Dop3, UD) triggers the synthesis of increasing leaf chlorophyll content, which in turn increases the apparent light-harvesting size, supporting an increased light absorption flux (ABS/RC). The higher ABS flux, however, was not matched by correspondingly higher TR or ET fluxes. Consequently, the excess energy flux which could not

be converted into photochemical reactions and was redirected toward the dissipation flux (DI) to protect the integrity of the photochemical apparatus [45,46]. This increased DI/RC flux corresponds to the energy lost in the activation of photoprotection/photoinhibition pathways which may explain the lower photosynthetic productivity recorded for the rocket crop grown at the highest PAR levels under the UD greenhouse. This result is in agreement with previously reported data on different plant species [42,47].

PMMA doping with rare earths did not influence either the brightness, the yellowness or the total chlorophyll content of rocket leaves (Table 4), while it increased the redness, reduced the greenness (Table 2) and it enhanced the antioxidant activity (ABTS and HAA), compared to the undoped control (Table 3). This could be interpreted as the response to a mild shade stress caused by the doped greenhouse covers. The contrasting results observed in the Chl content (Table 4) and the SPAD measurements of greenness (Figure 6) may be explained by considering that, even though the CIELAB colour space parameters can provide an estimate of leaf chlorophyll content, not always is a direct correspondence between these parameters found and the leaf colour results from the interaction of different factors including leaf anatomy, leaf morphology as well as pigment content rather than being solely determined by differences in leaf chlorophyll content. Indeed, Li et al. [48] reported that the different leaf colours of three cultivars (red, green, and mixed red and green) of *Alternanthera bettzickiana* resulted from the interaction between chloroplast morphology and different chlorophyll-to-anthocyanin ratios.

A significant interaction between the greenhouse cover and the time of harvest was found in the leaf concentration of phenols and total ascorbic acid (Figure 7). The values recorded under the clear and the doped PMMA were similar in the first three harvests, while they were higher in Dop3 and lower in Dop7 compared to control in the last one. As reviewed by Paradiso and Proietti [3] ascorbic acid as well as total phenol content in lettuce was found to respond to light spectral composition [49,50], while different secondary metabolic pathways are directly affected by radiation level, including ascorbate and phenolics synthesis [3]. Therefore, the recorded variations in phenols and ascorbic acid in rocket leaves may result from the combined effects of light intensity and light spectrum modulation under the different greenhouse cover panels.

## 5. Conclusions

In our study, conducted in the winter–spring season in a Mediterranean climate, the cultivation of wild rocket under PMMA greenhouse cover doped with 3% photoluminescent rare earths gave the highest marketable yield and antioxidant quality compared to other treatments.

However, the different doping proportions of the PMMA panels also resulted in different shading effects, thus affecting the light intensity inside the greenhouses. In our experimental conditions, both the plant photochemical metabolism and crop productivity were found to be directly related to light intensity inside the greenhouse. Although these results did not allow us to distinguish between the combined effects of shading and light spectrum modulation, it is reasonable to expect that light modulation with these greenhouse coverings could prompt different crop responses in different seasons, depending on the prevailing light conditions. Hence, further studies are required to investigate the possibilities offered by greenhouse covering doped with blends of rare-earth elements as an innovative technology for improving crop performance as well as environmental and financial sustainability in agriculture.

**Author Contributions:** Conceptualization: R.P., E.C., I.D.M., P.M., S.C. and M.M.; methodology: L.O., M.R. and A.T.; software: L.O. and G.M.; validation: I.D.M. and P.M.; investigation and resources: E.C. and G.M.; data curation: L.O. and I.D.M.; writing original draft preparation: R.P. and S.C.; writing—review and editing: all the authors; supervision and project administration: M.M.; funding acquisition: L.B. and M.M. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The datasets generated for this study are available on request to the corresponding author.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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