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ORIGINAL ARTICLE

Effects of UV-B radiation on the morphology, UV-B absorbing compounds and photosynthetic pigment content of *Plantago lanceolata* and *Rheum rhabarbarum*

Efectos de la radiación UV-B en la morfología, el contenido de compuestos absorbentes UV-B y pigmentos fotosintéticos de *Plantago lanceolata* y *Rheum rhabarbarum*

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ABSTRACT

In recent decades the fluence of ultraviolet-B (UV-B) radiation striking the ground has increased as a consequence of ozone depletion at southern latitudes. This phenomenon has sparked much interest in unravelling how plants acclimate to this condition. UV-B radiation triggers several responses that affect plant physiology, morphology and biochemistry. In this study, the effects of supplemental UV-B radiation on plant architecture, UV-B absorbing compounds and photosynthetic pigments were analyzed in two cosmopolitan species. A different range of responsiveness was found in the morphological parameters (leaf area, length and width; plant height) of species. The effects on photosynthetic pigment content and the UV-B absorbing compounds were studied through absorbance measurements. The obtained data showed that only carotenoids increased in irradiated plants of *R. rhabarabarum*. Likewise, an increase in the UV-B absorbing compounds content was found in irradiated plants from the same species. This effect may be regarded as a chemical protection against the damaging effects of light in photosynthesis and against oxidative cell damage. UV-B radiation induced photomorphogenic responses. In fact, all the morphological parameters of *P. lanceolata* were increased. The clear increase shown in leaf size and plant height suggest that cell division and stem elongation and/or differentiation processes are stimulated by UV-B radiation. These changes can be seen as an eustress effect and may indicate that plants are physiologically adapted to this level of UV-B radiation.

Keywords: distress, eustress, photomorphogenesis, UV-B effects.

RESUMEN

En las últimas décadas, la fluencia de radiación ultravioleta-B (UV-B) que golpea el suelo ha aumentado como consecuencia del adelgazamiento de la capa de ozono en las latitudes del sur. Este fenómeno ha despertado mucho interés en desentrañar cómo las plantas se aclimatan a esta condición. La radiación UV-B desencadena varias respuestas que afectan la fisiología, morfología y bioquímica de las plantas. En este estudio, se analizó el efecto de la radiación UV-B suplementaria en la arquitectura de la planta, los compuestos absorbentes de UV-B y los pigmentos fotosintéticos en dos especies cosmopolitas. Se encontró un rango diferente de capacidad de respuesta en los parámetros morfológicos (área foliar, longitud y ancho de las hojas; altura de la planta) de las especies. El efecto sobre el contenido de pigmentos fotosintéticos y los compuestos absorbentes de UV-B se estudió a través de mediciones de absorbancia. Los datos obtenidos mostraron que sólo los carotenoides aumentaron en plantas irradiadas de *R. rhabarabarum*. Asimismo, se encontró un aumento en el contenido de compuestos absorbentes de UV-B en plantas irradiadas de la misma especie. Este efecto puede considerarse como una protección química contra los efectos dañinos de la luz en la fotosíntesis y contra el daño celular oxidativo. Asimismo, también se encontraron respuestas fotomorfogénicas inducidas por la radiación UV-B. De hecho, todos los parámetros morfológicos de *P. lanceolata* se incrementaron. El claro aumento mostrado en el tamaño de la hoja y la altura de la planta sugiere que la división celular y los procesos de elongación y/o diferenciación del tallo son estimulados por la radiación UV-B. Estos cambios pueden verse como un efecto de "eustrés" y pueden indicar que dichas plantas están fisiológicamente adaptadas a este nivel de radiación UV-B.

Palabras clave: efectos UV-B, "distress", "eustress", fotomorfogénesis.

INTRODUCTION

The thinning of the ozone layer as a result of the increase in CFCs released into the atmosphere has remarkably increased the amount of UV-B radiation reaching the ground (Barnes *et al.* 2019). This high-energy radiation can be considered as a serious threat to the biosphere. It has led to major concerns about the effects of UV radiation on living organisms. UV-B radiation (280-320 nm) is an important environmental factor that regulates several plant processes. It initiates a complete suite of responses in plants that include plant physiology, morphology and biochemistry. Additionally, alterations to the leaf surface structure and leaf growth (Fina *et al.* 2017), increased levels of UV-B absorbing vacuolar pigments (Beggs *et al.* 1985; Cuadra *et al.* 1997) and changes in surface flavonoids (Cuadra & Harborne 1996) have been reported.

Since the late 1950s´ several reports have described the effects of UV-B radiation on plant architecture including anatomical and morphological features. After Brodführer (1955), who reported changes in plant architecture of *Arabidopsis thaliana* induced by solar UV radiation, diverse plant responses to UV-B radiation can be found in the literature. It is well known that in sensitive plants, stem height and leaf area, among other variables are reduced by UV-B exposure to different extents depending on plant species and cultivar (Lydon *et al.* 1987; Krizek *et al.* 1994). Furthermore, Teramura & Sullivan (1987) have shown that the relative growth is dependent on the plant growth stage. The most effective period occurs during the transition between vegetative and reproductive stages. Working on barley seedlings Tevini *et al.* (1981) found that the plant height is affected in a UV-B fluence-dependent manner. Similar results were found in *Phaseolus vulgaris* by Dumpert & Knacker (1985). Other growth studies with soyabean (Tevini *et al.* 1991) and cucumber seedlings (Tevini *et al.* 1993) also revealed that UV-B reduces plant height, leaf area and total dry weight. Typically, stems become shorter as reported for various species (Barnes *et al.* 1990; Hofmann & Campbell 2011). Alterations in leaf shape and decreases in leaf size (Cuadra *et al.* 2010), shorter petioles and leaf curling (Jansen 2002; Hectors *et al.* 2010) are among the most frequent recorded UV-B effects. Despite all this experimental evidence relating to reductions or deleterious effects of UV-B radiation on plant growth, Tezuka *et al.* (1993) reported increased growth in tomato (*Lycopersicum esculentum*) and radish plants. It seems that solar UV radiation encouraged stem growth in the later growth stages. Similarly, Zu *et al.* (2010) reported an increase in some morphological features of *Taxus chinensis* exposed to supplemental UV-B radiation. The lack of effects in plant architecture have also been reported (Germ *et al.* 2016). It would appear that all this variability in plant responses can be related to different plant phenotypes (Jansen *et al.* 2017).

In plants, enhanced levels of UV-B radiation cause stress and the most frequent response is the production of UV-B absorbing compounds. Several authors (Wellman 1985; Middleton & Teramura 1993) agreed that accumulation of these type of metabolites is a signal of UV-B damage. These compounds can act as sunscreens and provide chemical protection as a result of their absorptive properties. Moreover, some flavonoid derivatives have also been involved in phytohormones interactions which result in morphological alterations (Peer & Murphy 2007, Mahajan *et al.* 2011). These changes are displayed in some *Arabidopsis* mutants (Yin *et al.* 2014). On the other hand, phytochrome and other photoreceptors have also been involved in these interactions (Jansen 2002). It seems that UV-B radiation induced changes in cells that allow phytochrome to perform its photomorphogenic role. Genes from their biosynthetic pathways are also activated. For instance, Cuadra *et al.* (2020) analysed the expression profile of DaCHS1gen in response to UV-B radiation. CHS and other enzymes of the flavonoid pathway were likewise expressed after UV-B treatment (Xu *et al.* 2011).

There are many studies about the effect of supplemental UV-B radiation on light harvesting complexes. Most of them show inconsistent results in the relative change in the constituent photosynthetic pigments. These differences have been attributed to the different experimental conditions, cultivars (Middleton & Teramura 1993, Adamse & Britz 1992), PPFD treatments (Krizek 2004, Jordan *et al.* 2016) among others. In this context, Tevini *et al.* (1981) reported that chlorophyll content was decreased in bean seedlings and in barley seedlings. Carotenoids were also affected but less damaged than chlorophylls. A similar decrease in the chlorophyll *a* content was reported by Cuadra *et al.* (2004) in the Patagonian *Jaborosa magellanica*. Similar deleterious effects of UV-B radiation on the photosynthetic pigments content were found by Mosadegh *et al.* (2019) in sweet basil plants. Conversely, Adamse & Britz (1992) reported that the content of chlorophyll *a* and *b* increased under UV-B radiation in two cultivars of *Cucumis sativa*. An increase in chlorophyll content was also recorded by Alenius *et al.* (1995) in leaf discs of *Brassica napus*. In an outdoor experiment, Ibañez *et al.* (2008), working on *Citrus* plants reported that chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were higher in treated leaves although these changes depend on the developmental stage of plants. More recently in 2021, Wang reported an increase in the chlorophyll *a*, chlorophyll *b* and carotenoids content among other variables such as Hill reaction activity, photochemical quenching coefficient (qP), and Rubisco activity. All these contradictory results show that UV-B radiation can act as a 'eustress' or a 'distress' in plants (Hideg *et al.* 2013, Mosadegh *et al.* 2019). Alternatively, the wide range of responsiveness found between different cultivars and species may play an important role in this apparent variation.

In this paper we report the effects of UV-B radiation on the morphological features, UV-B absorbing compounds and photosynthetic pigment content of two plants: *Plantago lanceolata* L. ("ribwort plantain") and *Rheum rhabarbarum* L. ("rhubarb").

MATERIALS AND METHODS

Plant material

Seeds of *P. lanceolata* and *R. rhabarbarum* were collected from the country side surrounding Punta Arenas city in the Magellan´s Region and kept in the seed bank of the Chilean Agricultural and Livestock Service (SAG in Spanish). Seeds were kept at -18° C and 5% R.H. until the experiment started and then were sown on several plastic trays, containing the following soil mixture: 6,000 cc of perlite + 6,000 cc of vermiculite + 6,000 cc of ANASAC leaf earth, 6,000 cc of peat + 2 kg of ANAVIT guano. After 30-35 days' growth, plants were transplanted into individual plastic pots, filled with the same compost mixture (200 cc) and were then moved into the UV-B chamber the following day. Plants were watered every 4 days and ambient daytime temperatures inside the UV-B chamber were 24-30 °C and relative humidity 38-42%. For all measurements, leaf samples were taken from leaves developed in an enhanced UV-B ambient.

Light sources

In addition to greenhouse sunlight (PAR: 1200 μmolm^{-2} s^{-1}), UV-B radiation doses given to plants were supplied by using the same lamps and filter systems reported by Cuadra *et al.* (2004). Previous to use lamps and filters were treated and changed as indicated in Adamse & Britz (1992).

UV treatments

Plants were continuously exposed to 9 h of daily UV-B irradiation (similar to natural amount of sunlight on summer days) and harvested at different times of exposure. Lamps were placed in a mobile rack and hung at 0.70 m above the plants. This distance was chosen because it produces a daily UV-B irradiance of 1.5 Wm^{-2} nm which is similar to average doses received in Punta Arenas in the spring summer period. After irradiation the UV absorbing films (positioned to separate control and treated groups) were removed in order to give the same light conditions to all plants.

Radiation measurements

The spectral irradiance levels received by plants below the lamps were measured with the same spectrophotometer used in Cuadra *et al.* (2022). The spectrophotometer (Brewer MKIII, Kipp & Zonen) was calibrated using a NIST traceable 1000W tungsten filament quartz halogen lamp.

Experimental design

The experimental pattern used in this study corresponded to a randomized complete block design with several variables. 160 plants (30-35 days old) were distributed in two groups inside the experimental chamber: control group (-UVB) and treated group (+UVB). A UV absorbing plastic film was used to isolate both groups. Pot positions were randomized within each group every two days to minimize position effects. The lamp rack height was adjusted once a week to maintain the

UV irradiance levels.

UB-B absorbing compounds

Quantitation of internal UV-B absorbing compounds was carried out following the procedure of Caldwell (1968) and Mirecki & Teramura (1984). Internal phenolics were analyzed from leaves from the top half of plants (2 leaves from 16 different plants; each species). 1 cm^2 leaf disks were ground using a pestle and mortar with 2 ml of MeOH:H_2O:HCl=79:20:1 (v/v). Homogenates, combined with further washing of the pestle and mortar with 1 ml of the same solvent mixture, were centrifuged (Heraeus, Labofuge 200) at 3,000 rpm for 10 min. Supernatants were then filtered (Whatman N° 1) and evaporated to dryness at 40 °C. Residues were redissolved in MeOH.

Photosynthetic pigments

Several leaf samples from the top half of plants were analyzed (2 leaves from 16 different plants; each species). Total chlorophylls and carotenoids were extracted from individual leaf disks (1 cm^2) following the procedure indicated in Hiscox & Israeltam (1979). Absorbance was determined at 664, 648 and 470 nm on 1 ml of samples and the absorbance spectrum recorded between 200 and 700 nm (Shimadzu UV-160A spectrophotometer). Photosynthetic pigment concentrations were calculated according to equations given in Chapelle *et al.* (1992) by using the absorbance measurements: 664 nm (for CHl*a*), 648 nm (for CHl*b*) and 470 nm (for carotenoids).

Growth variables

Leaf area of adaxial epidermis and leaf length were determined by a portable leaf area meter (Bioscientific ADC, AM300-02). Stem elongation (plant height) was measured from soil (pot) level to the top of the plant. Leaves used in area and length measurements were collected from the top half of plants (first stage). Each harvest was carried out on 16 different plants.

Statistical analysis

Data of leaf area, leaf length, plant height, UV-B absorbing compounds and photosynthetic pigments were analyzed using procedures for a randomized complete block design. Statistical assessments (ANOVA, LSD test) were performed for all absorbance measurements using the Statgraphics Centurion XVI Statistical Package.

RESULTS

Internal UV-B absorbing compounds

The UV spectra of MeOH leaf extracts showed two major absorption peaks at 272, 347 and a minor peak at 420 nm (*R. rhabarbarum*) and peaks at 286 and 331 nm (*P. lanceolata*) among others (Fig. 1 and 2). TLC analysis of these extracts showed 2-3 spots (Rf = 0.79 and 0.62), with a light blue fluorescence under UV/NH_3 that is typical of hydroxycinnamic esters.

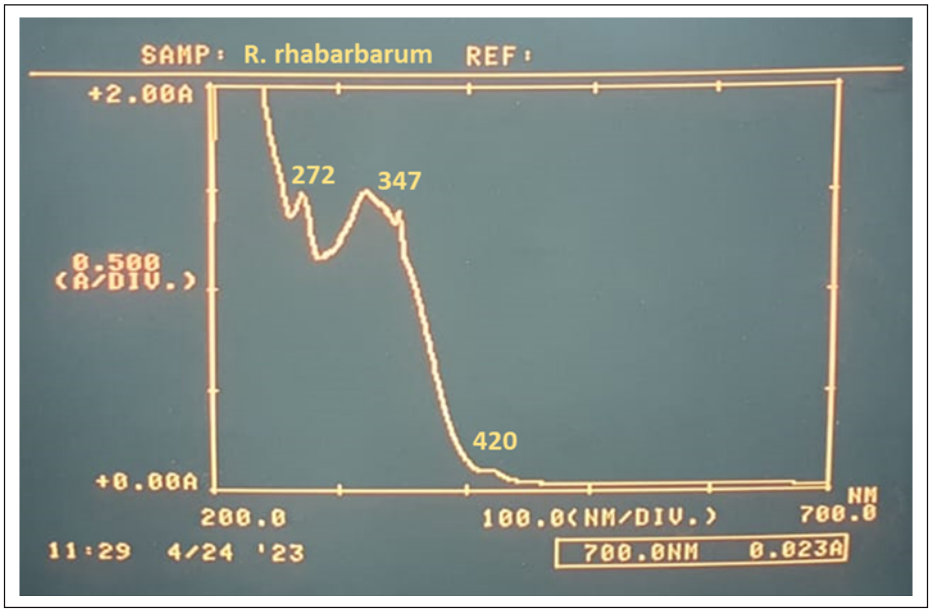

Figure 1. UV-VIS spectrum of MeOH:H_2O:HCl=79:20:1 (v/v) extract of *R. rhabarbarum*. It shows two peaks at 272, 347 and 420 nm. / Espectro UV-VIS del extracto de MeOH:H2O:HCl=79:20:1 (v/v) de *R. rhabarbarum*. Se observan señales a 272, 347 y 420 nm.

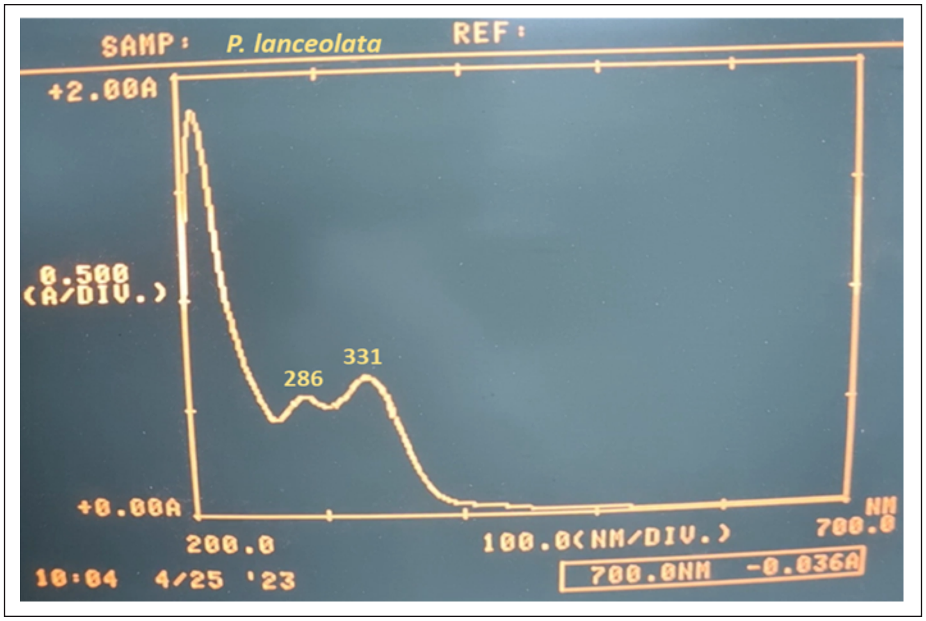

Figure 2. UV-VIS spectrum of $MeOH:H_2O:HCl$=79:20:1 (v/v) extract of *P. lanceolata*. It shows two peaks at 286 and 331 nm. / Espectro UV-VIS del extracto de $MeOH:H_2O:HCl$=79:20:1 (v/v) de *P. lanceolata*.. Se observan dos señales a 286 y 331 nm.

In order to compare the effects of UV-B radiation on the internal UV-B absorbing compounds (flavonoids and hydroxycinnamic esters) on both plant species, quantitation was carried out by measuring the MeOH extracts absorbance at 300 nm. Table 1 shows that treated *R. rhabarbarum* plants have a higher absorbance than non-irradiated plants and that the differences between internal UV-B absorbing pigments in both plant groups are statistically significant after 31 days of treatment when the absorbance of treated plants is 71% higher than in controls. Table 2 shows that irradiated plants of *P. lanceolata* have higher absorbance at 300 nm but these differences are not statistically significant.

Photosynthetic pigments

Different responses are observed in both plant species. Similarly, the effect on photosynthetic pigments varies among pigment types. There are no statistical differences in the chlorophyll *a*, chlorophyll *b* content of *R. rhabarbarum* while carotenoids displayed higher values in treated plants compared with controls (20.4% of increase; Table 3). On the other hand, the UV-B treatment did not affect the photosynthetic pigment content of *P. lanceolata* (Table 4).

Growth

In addition to the effects on the photosynthetic pigments, other important physiological processes determining morphology and plant growth were positively and adversely affected by UV-B radiation (Tables 5 and 6). As in the photosynthetic pigment content diverse responses were found. For instance, in *R. rhabarbarum* only plant height was significantly affected by the UV-B treatment (27.8% of decrease; Table 5). Although there was a decrease in the other variables these differences were not significant. On the other hand, in *P. lanceolata* a clear increase was displayed in all the morphological parameters of irradiated plants compared to controls. Table 6 shows that leaf area was increased by up to 59.9%, leaf length, leaf width and plant height increased by about 30.8%, 24% and 30.9% respectively. All these differences were statistically different.

Table 1. Effect of UV-B radiation on the internal UV-B absorbing compounds content of *R. rhabarbarum*. $(A_{300})^{a,b}$. / Efecto de la radiación UV-B en el contenido de los compuestos internos absorbentes de UV-B de *P. lanceolata* (A300)^{a,b}.

Control	Treatment	F[c]	P[d]
0.489 ± 0.105a	0.836 ± 0.105b	5.49	0.0344

[a] Mean concentration (AU; N=16) and standard error of internal UV-B absorbing compounds, after 31 days of treatment. Means followed by different letters are statistically significant at $P<0.05$ level, as determined by LSD in ANOVA.
[b] Degrees of freedom=1; F and P (determined in ANOVA) are given for UV-B absorbing pigments by treatment.
[c] F-value was determined by dividing the mean square between-groups by the mean square within-groups.
[d] Confidence level is 95%.

DISCUSSION

The clear increase observed in the absorbance (A_{300}) of *R. rhabarbarum* irradiated plants (Table 1) is a well-known plant response to UV-B radiation which is based on the absorption bands of hydroxy-cinnamic esters being within the UV-B range. Because of their UV absorption spectra (280-320 nm) these compounds are likely to participate in plant protection against UV-B radiation. In the same way, the accessory pigment content increased after the UV-B treatment. The higher values observed in the carotenoids content may be explained on the basis of its role in chlorophyll´s protection from photodestruction by quenching the Chl triplet and singlet oxygen states (Robertson *et al.* 1966, Young & Frank 1996). Zeaxanthin and xanthophyll have also been involved in quenching Chl excited molecules and in the transfer of energy to chlorophyll molecules (Schreiber & Neubauer 1990, Collini 2019).

Similar findings were reported by Middleton & Teramura (1993,1994). They correlated the carotenoid content with UV-B absorbing compounds among other physiological processes. These plant responses indicate that some pigments are accumulated as a consequence of UV-B exposure and may be explained as a plant adaptive strategy. This chemical protection against damaging effects of light in photosynthesis and against the oxidative cell damage it has also been studied by Zia-Ul-Haq (2021). Perhaps, this defensive mechanism may decrease leaf penetration and help plants to dissipate some of this harmful radiation away from the reaction center (PS II) and other inner tissues via greater absorption (greater concentration) of UV-B absorbing compounds. In this way, plants avoid irreparable damage to membranous systems (e.g., thylakoids) and important biological processes such as photosynthesis. The effects of UV-B radiation in plant morphogenesis involve changes in cell division, stem elongation, microtubules organization and others (Ma *et al.* 2017). According to our findings, UV-B radiation induced a negative effect of in all the morphological variables of *R. rhabarbarum* but only the differences in plant height are statistically significant. Similar reductions in stems (Cuadra *et al.* 2010, Pandey *et al.* 2022, Ramamoorthy *et al.* 2022), seedlings (Tevini *et al.* 1991, Gao *et al.* 2019) and petioles (Jansen 2002) have been reported in the literature.

Table 2. Effect of UV-B radiation on the internal UV-B absorbing compounds content of *P. lanceolata*. (A_{300})^{a,b}. / Efecto de la radiación UV-B en el contenido de los compuestos internos absorbentes de UV-B de *P. lanceolata* (A_{300})^{a,b}.

Control	Treatment	Fc	Pd
0.832 ± 0.080a	0.852 ± 0.080a	0.03	0.8610

[a]Mean concentration (AU; N=16) and standard error of internal UV-B absorbing compounds, after 31 days of treatment. Means followed by different letters are statistically significant at P<0.05 level, as determined by LSD in ANOVA.

[b]Degrees of freedom=1; F and P (determined in ANOVA) are given for UV-B absorbing pigments by treatment.

[c]F-value was determined by dividing the mean square between-groups by the mean square within-groups.

[d]Confidence level is 95%.

All these deleterious effects may be seen as a distress caused by the UV-B treatment. On the other hand, the photosynthetic and auxiliary pigment content, including the UV-B absorbing compounds of *P. lanceolata*, showed only slight differences between groups and these are not statistically significant. A very different response to UV-B radiation was observed in the morphological parameters of this species. As pointed out by Hideg *et al.* (2013), a sort of "eustress" effect is observed in the irradiated plants which showed an increase in all measurements. The clear increase shown in leaf size and plant height suggest that cell division and stem elongation and/or differentiation processes are stimulated by UV-B radiation. In this case, the absence of

Table 3. Effect of UV-B radiation on the photosynthetic pigments content of *R. rhabarbarum*[a]. / Efecto de la radiación UV-B en el contenido de pigmentos fotosintéticos de *R. rhabarbarum*[a].

Pigment	Control	Treatment	F	P
Chl*a*	5.06 ± 0.19a	5.60 ± 0.19a	4.11	0.0621
Chl*b*	2.83 ± 0.15a	3.031 ± 0.15a	0.86	0.3707
Carotenoids	0.79 ± 0.03a	0.951 ± 0.03b	13.65	0.0024

[a]Mean concentration (µg pigment/cm^2; N=16) and standard error of plant pigments. Means followed by different letters are statistically significant at P<0.05 level, as determined by LSD in ANOVA.

[b]Degrees of freedom=1; F and P (determined in ANOVA) are given for each plant pigment by treatment.

[c]F-value was determined by dividing the mean square between-groups by the mean square within-groups.

[d]Confidence level is 95%.

deleterious effects indicates that plants are physiologically adapted to this level of UV-B radiation. It seems that the UV-B doses given to plants may promote plant growth provided they do not exceed that found in solar UV spectrum (Tezuka *et al.* 1993). In this connection, the provenance of seeds collected under highly sun-exposed zone may partially explain these findings.

The range of responsiveness found between both species may play an important role in this variation. This positive effect on plant production is characteristic of "sun adapted" plants which are taller, with larger leaves, stems, and roots, and, therefore, total biomass (Middleton & Teramura 1994). In the present study, the positive photomorphogenic effects found on plant biomass as shown by plant height, leaf length, leaf width and leaf area of the irradiated plants cannot be explained on the grounds of an increase in photosynthesis because of the lack of response in the chlorophyll content. The eustress observed in *P. lanceolata* may indicate a more adapted condition for this plant species. This positive influence is displayed in the area, width and length of treated leaves. Plant height is also higher in irradiated plants.Similar increases in growth in *Lycopersicum esculentum* (Tezuka *et al.* 1993) and other morphological parameters in *Taxus chinensis* (Zu *et al.* 2010) have been reported. It seems that solar UV radiation encouraged stem growth in the later growth stages. However, these results do not agree with several reports on different crop species, *Cucumis sativa* (Tevini & Teramura 1989; Kryzek *et al.* 1994), *Glycine max* (Teramura & Sullivan 1987, Miles 1993), *Hordeum vulgare* (Tevini *et al.* 1981) and *Phaseolus vulgaris* (Cen & Bornman 1990) which recorded a reducing plant height and leaf area effect. This may be because the morphological changes depend on the fluence and wavelength of radiation used in the experiments and the different development period of plants being more sensitive during transition from vegetative to reproductive stage. In another approach, Jansen (2002) discussed that UV-B induced morphogenic responses may not involve a specific photoreceptor (e.g., UVR8) but changes in the flavonoid pathway can affect auxin transport and hence plant morphology. However, the lack of correlation between UV-B absorbing compounds and morphogenesis found in this study do not permit a contribution to this discussion.

UV-B radiation is an environmental signal that activates several plant protection mechanisms such as accumulation of phenolics induced by the expression of certain genes (Ibdah *et al.* 2002, Götz *et al.* 2010, Morales *et al.* 2010). In some cases, low fluence rates of short-wavelength UV (280-320 nm) induce photomorphogenic responses that affect plant architecture and regulate leaf growth, hypocotyl elongation (Jenkins 2009, Wargent *et al.* 2009). These

TABLE 4. Effect of UV-B radiation on the photosynthetic pigment content of *P. lanceolata*[a]. / Efecto de la radiación UV-B en el contenido de pigmentos fotosintéticos de *P. lanceolata*[a].

Pigment[b]	**Control**	**Treatment**	**F**[c,d]	**P**
Chl*a*	1.99 ± 0.11a	1.84 ± 0.11a	0.93	0.3518
Chl*b*	0.52 ± 0.04a	0.54 ± 0.04a	0.07	0.8015
Carotenoids	0.72 ± 0.04a	0.71 ± 0.04a	0.04	0.8516

[a]Mean concentration (µg pigment/cm^2; N=16) and standard error of plant pigments. Means followed by different letters are statistically significant at P<0.05 level, as determined by LSD in ANOVA.
[b]Degrees of freedom=1; F and P (determined in ANOVA) are given for each plant pigment by treatment.
[c]F-value was determined by dividing the mean square between-groups by the mean square within-groups.
[d]Confidence level is 95%.

TABLE 5. Effects of UV-B radiation on leaf area[a,b], leaf length[a,c], leaf width[a,d] and plant height[a,e] of *R. rhabarbarum*. / Efecto de la radiación UV-B en el área,[a,b], largo[a,c] y ancho[a,d] de las hojas y la altura[a,e] de la planta de *R. rhabarbarum*.

	Area	**Length**	**Width**	**Height**
Control	642.88 ± 53.00	53.28 ± 3.98	14.43 ± 1.26	11.95 ± 0.74
Treatment	549.88 ± 53.00	51.45 ± 3.98	11.91 ± 1.26	9.35 ± 0.74

[a]Mean area (cm^2; N=16), mean length (cm; N=16), mean width (cm; N=16) and mean height (cm; N=16) and standard error, after 28 days of treatment.
[b]Means are not statistically significant as determined by LSD in ANOVA: DF=1; F=1.54; P<0.2351.
[c]Means are not statistically significant as determined by LSD in ANOVA: DF=1; F=0.11; P<0.7507.
[d]Means are not statistically significant as determined by LSD in ANOVA: DF=1; F=2.00; P<0.1789.
[e]Means are statistically significant as determined by LSD in ANOVA: DF=1; F=6.26; P<0.0253.

UV-B photomorphogenic responses are mediated by the UV-B photoreceptor (UVR8; Rizzini *et al.* 2011). In recent years, the understanding of these morphological changes in plants, mediated by the UVR8, has increased and hormonal interactions have been demonstrated (Hayes *et al.* 2014).

In this context, gibberellic acid and auxin metabolism have been involved in these interactions. According to Morales *et al.* (2010), the UV-B-induced phenotype exists and some of its architectural features are UVR8 mediated. It seems that *P. lanceolata* is one of this kind of UV-B phenotype as suggested by the positive effects on all the morphological parameters and the lack of response in the photosynthetic pigments found in this study. It would appear that these are resistant plants species which can tolerate the levels of UV-B radiation given. It seems that their capacity to dissipate excess energy is sufficient and they do not need to activate other mechanisms to deal with these levels of radiation. As pointed out by Qi *et al.* (2003) and Klem *et al.* (2012), the changes induced by UV-B radiation in plant height and in the concentration of protective flavonoids of *R. rhabarbarum* plants may be considered as co-occurring phenomena.

Accumulation of flavonoids and hydroxycinnamic esters is a very efficient biosynthetic process. These compounds act as sunscreens giving protection against UV radiation. Experimental evidence has shown that UVR8 and other photoreceptors participate in this environment-plant interaction. Polar auxin transport and auxin catabolism may also be involved in these plant responses. However, the underlying mechanisms are not well understood yet and integrated approaches could help with a full understanding.

Table 6. Effects of UV-B radiation on leaf area[a,b], leaf length[a,c,] leaf width[a,d] and plant height[a,e] of *P. lanceolata*. / Efecto de la radiación UV-B en el área[a,b], largo[a,c] y ancho[a,d] de las hojas y la altura[a,e] de la planta de *P. lanceolata*.

	Area	Length	Width	Height
Control	9.32 ± 0.66	8.86 ± 0.46	1.04 ± 0.03	9.31 ± 0.53
Treatment	14.90 ± 0.66	11.59 ± 0.46	1.29 ± 0.03	12.19 ± 0.53

[a] Mean area (cm^2; N=16), mean length (cm; N=16), mean width (cm; N=16) and mean height (cm; N=16) and standard error, after 28 days of treatment.

[b] Means are statistically significant as determined by LSD in ANOVA: DF=1; F=36.27; P<0.0000.

[c] Means are statistically significant as determined by LSD in ANOVA: DF=1; F=17.60; P<0.0009.

[d] Means are statistically significant as determined by LSD in ANOVA: DF=1; F=26.42; P<0.0002.

[e] Means are statistically significant as determined by LSD in ANOVA: DF=1; F=14.49; P<0.0019.

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