



Morphological, biochemical and physiological responses of Indian cress (*Tropaeolum majus*) to elevated UV-B radiation

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Abstract

Background and Purpose: UV-B radiation is an important environmental factor for many plants with remarkable influence on defence-related secondary metabolite biosynthesis. Possible consequences of UV-B radiation on plants have been widely reported, but its effect on secondary plant metabolites in ornamental and medicinal plants is poorly understood. The aim of the present research was to establish whether it is feasible to cultivate *Tropaeolum majus* under conditions of enhanced UV-B radiation to alter the content of total phenolic compounds in leaves and flowers and whether UV-B treatment affect plant physiological response.

Material and Method: An outdoor study was conducted to examine the effect of different levels of UV-B radiation on the morphological (specific leaf area and length of internodes and petiole), biochemical (photosynthetic pigments, UV absorbing compounds) and physiological characteristics (photosynthesis, photochemical yield of PSII, transpiration rate, water use efficiency) of the widely cultivated annual herb Indian cress (*T. majus* L.).

Results and Conclusions: Enhanced UV-B radiation induced increased synthesis of total phenolic compounds in leaves, but not in flowers. Photosynthesis and photochemical yield of PSII were mostly unaffected by UV-B. Transpiration rate was higher at elevated UV-B levels in the beginning and peak of the season. Specific leaf area and length of internodes and petiole were unaffected. UV-B treated specimens of *T. majus* possessed enhanced amounts of total phenolic compounds, which are important for utilisation of *T. majus* herbs for human health. Enhanced UV-B treatment affected flowering of *T. majus* at the end of the growing season, which may have an important negative implication for success of this species in elevated UV-B radiation environments.

INTRODUCTION

Increasing UV-B radiation, resulting from air pollution-induced ozone depletion, has raised awareness of the effects of UV-B on the ecosystem (1). In spite of current efforts to restrict the production of ozone-depleting substances, thinning of the stratospheric ozone layer, with increased penetration of ultraviolet-B (UV-B) radiation to the earth's surface, will continue for decades (2). Recovery of the stratospheric ozone layer to conditions seen in 1979–1992 is not expected until 2040–2050 (3). UV-B stress

is one of the most important abiotic stress factors and can influence almost every aspect of plant physiology and biochemistry (4). Its effects on plants include inhibited growth, morphological changes and increased levels of phenolic substances (5, 6). The harmful effects of UV-B radiation on plants are often a consequence of the production of reactive oxygen species (ROS) (7), which eventually result in oxidative stress. The alleviation of oxidative damage caused by enhanced UV-B is often correlated with an effective antioxidant system in plants (4). Protective responses against enhanced UV-B radiation include increased production of UV-B absorbing and protective compounds: flavonoids, phenylpropanoids, and carotenoids (8). The UV-B absorbing compounds, particularly carotenoids and phenolic compounds, act as screening pigments (9) thereby are protecting the photosynthetic tissues. Multiple functions of flavonoids are well known (10, 11). It was shown by Rojas-Lillo et al. (12) that in highbush blueberry (*Vaccinium corymbosum*) cultivar Brigitta, manganese and UV-B radiation treatment induced an increased concentration of photoprotective compounds and thus enhanced resistance to oxidative stress.

The potential consequences of UV-B radiation on plants have been widely studied, but there is limited understanding of its effects on secondary plant metabolites on plants grown for horticulture, food and medicinal properties. *Tropaeolum majus* (Indian cress), which originates from the Andes in Bolivia, deserves special attention since it is grown widely as a medicinal plant, for human food, and as an ornamental plant (13, 14, 15). It has been consumed in salad or soup for decades (13). In natural medicine, *T. majus* is used to treat infections of the urinary tract (14). Herbs and flowers of *T. majus* contain high levels of flavonoids, which have apparent beneficial effects on human health (15, 16), attributed to their antioxidant activities (17).

The aim of the present research was to establish whether it is feasible to cultivate *T. majus* under conditions of enhanced UV-B radiation and whether the content of total phenolic compounds in leaves and flowers is altered. Specifically, it is important to determine whether solar and elevated UV-B radiation affect plant performance (photochemical yield of PSII, photosynthesis, transpiration rate, water use efficiency and photosynthetic pigments).

MATERIAL AND METHODS

Culturing of *Tropaeolum majus*

T. majus seeds were sown in May in a sandy soil in pots (15x15x15 cm) on an outdoor research plot (Botanical garden, University of Ljubljana: 320 m above sea level, 46°35'N, 14°55'E). After one month, seedlings were exposed to different treatments. Average monthly temperatures, cumulative monthly precipitation and hours of solar radiation during the experiment are indicated in the Table

1. Samples for the analysis of plants grown under each treatment were chosen randomly out of 100 specimens.

Table 1. Average monthly temperatures, cumulative monthly precipitation and hours of solar radiation during the experiment

Month	June	July	August	September
Temperature (°C)	21.1 ± 3.8	21.3 ± 2.0	20.1 ± 1.9	15.0 ± 3.5
Precipitation (mm)	175.6	127.0	181.2	87.5
Solar radiation (h)	9.9 ± 5.0	8.9 ± 3.3	7.1 ± 3.5	5.9 ± 3.8

UV-B supplement system

A UV-B supplement system was designed as described by Björn and Teramura (18). (1) Simulation of 17% ozone depletion [UV-B(+)] was performed using Q-Panel UV-B 313 lamps, filtered with cellulose diacetate filters, which blocks the UV-C range (wavelengths lower than 280 nm). (2) In control plot plants were exposed to the radiation produced by Q-Panel UV-B 313 lamps filtered with Mylar foil which cuts off wavelengths below about 320 nm (19), therefore supplemental UV-A radiation (with potential beneficial effects) and natural UV-B radiation were included in the treatment. In both treatments (1,2), a panel of 12 UV lamps (Q-Panel UV-B 313 lamps) was positioned 1m above the plants. (3) On the third plot [UV-B(-)], Mylar foil filter was positioned 80 cm above the plants to reduce cca. 60% of natural UV-B and UV-A radiation. Biologically effective UV-B (UV-B_{BE}) doses were calculated and adjusted weekly using the program of Björn and Murphy (20), based on the generalized plant action spectrum (21). Supplemental UV-B radiation corresponded to 17% ozone depletion, that presented from 35 to 58% increase of biologically active radiation UV-B_{BE}. That meant supplemental doses from 1.16 and 2.16 kJ m⁻² d⁻¹ respectively, depending on the day of the year (20). The time of the exposure was changing with the respect of the time of the year. Exposure lasted from June to end of September.

Biochemical analyses

Total chlorophyll (Chl) content and carotenoid content were determined as reported by Lichtenthaler (22). The basic procedure for total phenolic compounds (methanol soluble UV-B and UV-A absorbing compounds) followed the method described by Mirecki and Teramura (23). UV-absorbing compounds (UV AC) were extracted from freshly homogenised plant material (approx. 0.1 g DM) with methanol:distilled water:HCl = 79:20:1 (v/v/v). Samples were then centrifuged in a top refrigerated ultracentrifuge (5,000 Hz, 10 °C, 10 min) and the extinction of supernatants measured in the range from 280–400 nm at intervals of 1 nm with UV/VIS Spectrometer System. Absorbances from 280–320 nm for UV-B and from 320–400 nm for UV-A absorbing compounds were integrated and expressed per dry mass (DM) of the sample.

Measurements of photosynthesis, transpiration rate and photochemical yield of PSII

Light-saturated net photosynthesis rate (Pn) was measured with a portable infrared gas analyser (LI-6200, LI-COR, Lincoln, NE, USA) and transpiration with a porometer (LI-1600, LI-COR, Lincoln, NE, USA). Water use efficiency was calculated as the ratio of photosynthesis to transpiration (24).

Photochemical yield of photosystem PSII was determined by fluorescence measurements. This is a non-intrusive method that allows rapid assessment of quantum yield of electron flow through PSII. Measurements were carried out with a portable fluorometer (OS-500, Opti-Sciences, Tyngsboro, MA, USA). The potential quantum yield of PSII ($F_v/F_m = (F_m - F_0)/F_m$) quantifies the maximum efficiency of the primary photochemical events in photosynthesis. F_0 and F_m are the minimal and maximal chlorophyll *a* fluorescence yields in dark adapted samples, and F_v is the variable fluorescence. Fluorescence was excited with a saturating beam of “white light” [photosynthetic photon flux density (PPFD) = 8 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s]. The effective quantum yield of PSII was measured under saturating irradiance by providing a saturating pulse of “white

light” (PPFD = 9 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s), using a standard 60° angle clip. The effective quantum yield of PSII, given by formula $(F_m' - F)/F_m' = \Delta F/F_m'$, gives the actual efficiency of energy conversion in PSII (25). F_m' is the maximum fluorescence signal of an illuminated leaf after a pulse of saturating light and F is the steady state fluorescence (26).

All gas exchange and fluorescence measurements were made each day between 11.00 h and 15.00 h (local time) (PPFD \geq 1100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at ambient temperature and CO_2 concentration throughout the experiment.

Morphological analyses

Specific leaf area, lengths of petiola and of internodes were recorded for all treated and untreated plants at the end of the season.

Statistical analyses

SPSS was used for ANOVA (One-way, and multifactor) analysis. The significance of the differences ($p \leq 0.05$) was tested with post hoc Least Significant Difference (LSD) test after the analysis of variance. When parametric analysis was not admissible, Kruskal Wallis test was applied.

Table 2: Content of chlorophyll *a* and *b* and carotenoids per dry mass, net photosynthetic rate, transpiration rate, potential and effective quantum yield of PSII, UV-A absorbing compounds in leaves and flowers in *Tropaeolum majus* under reduced level of UV-B radiation - UV-B(-), ambient radiation - control and elevated UV-B radiation - UV-B(+) with time. Mean values \pm SD (standard deviation), $n = 5$.

Months	June			August			September		
	Treatment								
Parameter	UV-B(-)	Control	UV-B(+)	UV-B(-)	Control	UV-B(+)	UV-B(-)	Control	UV-B(+)
Chl <i>a</i> (mg/g DM)	2.58 ^a ± 0.74	3.53 ^b ± 0.52	2.11 ^a ± 0.71	5.36 ^a ± 0.65	4.98 ^a ± 0.72	5.35 ^a ± 0.62	5.69 ^a ± 0.54	5.77 ^a ± 0.91	4.79 ^a ± 1.01
Chl <i>b</i> (mg/g DM)	1.73 ^a ± 0.45	2.29 ^b ± 0.36	1.36 ^a ± 0.45	3.46 ^a ± 0.39	3.22 ^a ± 0.51	3.43 ^a ± 0.44	3.35 ^a ± 0.31	3.41 ^a ± 0.56	2.85 ^a ± 0.65
Car (mg/g DM)	0.70 ^a ± 0.14	0.89 ^a ± 0.09	0.54 ^a ± 0.15	1.11 ^a ± 0.10	1.05 ^a ± 0.12	1.22 ^a ± 0.13	1.44 ^a ± 0.14	1.57 ^a ± 0.22	1.31 ^a ± 0.20
Pn ($\mu\text{mol CO}_2/\text{m}^2\text{s}^{-1}$)	0.26 ^a ± 0.12	0.30 ^a ± 0.18	0.50 ^a ± 0.20	0.54 ^a ± 0.14	0.51 ^a ± 0.19	0.62 ^a ± 0.14	0.51 ^a ± 0.13	0.62 ^a ± 0.09	0.47 ^a ± 0.06
Tr (mol (H ₂ O)/m ² s ⁻¹)	0.36 ^a ± 0.15	0.48 ^{ab} ± 0.09	0.69 ^b ± 0.19	0.25 ^a ± 0.07	0.24 ^a ± 0.07	0.39 ^b ± 0.05	0.12 ^a ± 0.04	0.16 ^a ± 0.02	0.13 ^a ± 0.02
F_v/F_m (rel. units)	0.75 ^a ± 0.06	0.77 ^a ± 0.07	0.81 ^a ± 0.02	0.83 ^a ± 0.01	0.79 ^a ± 0.03	0.81 ^a ± 0.05	0.81 ^a ± 0.02	0.83 ^a ± 0.01	0.83 ^a ± 0.01
$\Delta F/F_m'$ (rel. units)	0.50 ^a ± 0.15	0.43 ^a ± 0.08	0.40 ^a ± 0.08	0.50 ^a ± 0.06	0.37 ^b ± 0.05	0.37 ^b ± 0.10	0.46 ^a ± 0.07	0.46 ^a ± 0.05	0.44 ^a ± 0.07
UV-A AC - leaves (rel. units/DM)	626 ^a ± 73	856 ^b ± 130	1171 ^b ± 152	853 ^a ± 226	1500 ^b ± 310	950 ^a ± 98	nm	nm	nm
UV-A AC - flowers (rel. units/DM)	1249 ^a ± 116	1161 ^a ± 426	1196 ^a ± 374	1990 ^a ± 533	nm	nm	nm	nm	nm

Legend: chl - chlorophyll, car - carotenoids, Pn - Net photosynthetic rate, Tr - transpiration rate, F_v/F_m - potential quantum yield of PSII, $\Delta F/F_m'$ - effective quantum yield of PSII, nm - no measurement. Different letters indicate significant difference among values within each sampling at $p \leq 0.05$ confidence level.

RESULTS AND DISCUSSION

UV-B radiation corresponding to 17% reduction of the ozone did not exert any effect on levels of chlorophylls or carotenoids in *T. majus* (Tab. 2). However the chlorophyll *a* and *b* contents in June were the highest in control treatment that may be related to supplemental UV-A radiation that had beneficial effect on DNA photo-repair mechanisms (27). Absence of the effect of UV-B radiation on the amount of chlorophylls or carotenoids in other months of experiments could be a consequence of the rapid growth of the plant, which is also the case in some agricultural plants. Little effect of UV-B on chlorophyll content was observed in *Phaseolus vulgaris* (28) or *Helianthus annuus* (29) plants. In the study of Mohammed and Tarpley (30), none of the rice cultivars showed changes in levels of carotenoids (UV-B screening pigment) with increased UV-B levels. Similar results were observed for soybean (*Glycine max*) exposed to UV-B radiation (8). On the other hand, exposure of *Prunus dulcis* plants to UV-B led to a substantial reduction in the content of both Chl *a* and Chl *b* (31), which reveals possible damage to the photosynthetic capacity of the chloroplasts (32). Mackerness et al. (5), who studied the effect of UV-B radiation on buds of pea, suggested that under UV-B stress, plants sacrifice their chloroplasts in order to protect the rest of the cell. UV-B radiation might affect the photosynthetic pigments, either through inhibition of their synthesis or effects on the enzymes involved in the chlorophyll biosynthetic pathway (33).

Levels of UV-B and UV-A absorbing phenolic compounds in leaf increased in response to UV-B radiation in *T. majus* during the period of intensive growth (Fig. 1, Tab. 2). The majority of primary producers respond to UV-B radiation by producing UV-absorbing compounds that provide a protective screen that filters out the UV-B (34). An increase in UV-B absorbing compounds accom-

panying an increase in UV-B level was observed for rice cultivars (35), St John's wort (36), common and Tartary buckwheat (37), lettuce (38), *Gnaphalium luteo-album* (39), and highbush blueberry leaves (40).

In another study, Kunz et al. (41) explained that plants counteract cell damage by attenuating the UV dose received, by accumulating UV-absorbing secondary metabolites that neutralize reactive oxygen species (ROS) produced by UV radiation. Likewise, Qaderi et al. (42) reported that enhanced UV-B radiation increased the amount of UV-B absorbing compounds at epicuticular wax. Albert et al. (43) found that plants from high level UV-B environments contain more UV-B absorbing compounds than those under lower UV-B treatment. In desert, UV radiation stimulated the biosynthesis of UV absorbing compounds in annual plants, performing a photoprotective function (44). Our results suggest that UV-B level influences the accumulation of secondary metabolites in *T. majus* and thus, by regulating growth conditions, it is possible to influence the concentrations of substances and the quality of *T. majus* herb. Amounts of these substances in *T. majus* are comparable to those in common and Tartary buckwheat (37). Buckwheat species originate from high altitude areas and it is well known that they contain high levels of UV absorbing compounds (45, 46, 47, 48). *T. majus* originates from the Andes mountains (South America), where enhanced UV-B levels are expected.

The amount of UV absorbing compounds in flowers was similar at all UV-B levels. However, experimental plants ceased to flower in September when exposed to ambient and enhanced UV-B radiation, although they were still in flower under the reduced levels of UV-B radiation (Fig. 2, Tab. 2). These observations indicate the sensitivity of flower initiation in *T. majus* to enhanced and even ambient levels of UV-B solar radiation. Enhanced UV-B radiation can accelerate or retard the time of flowering (49).

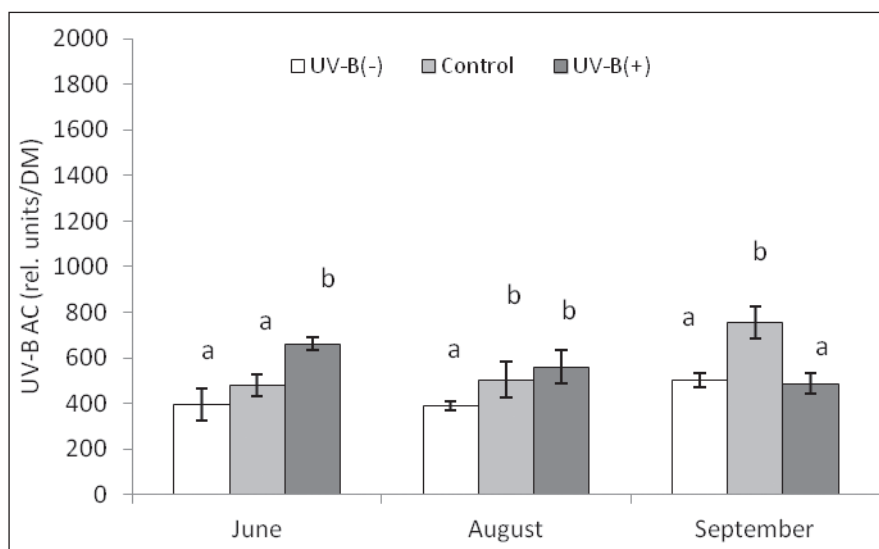
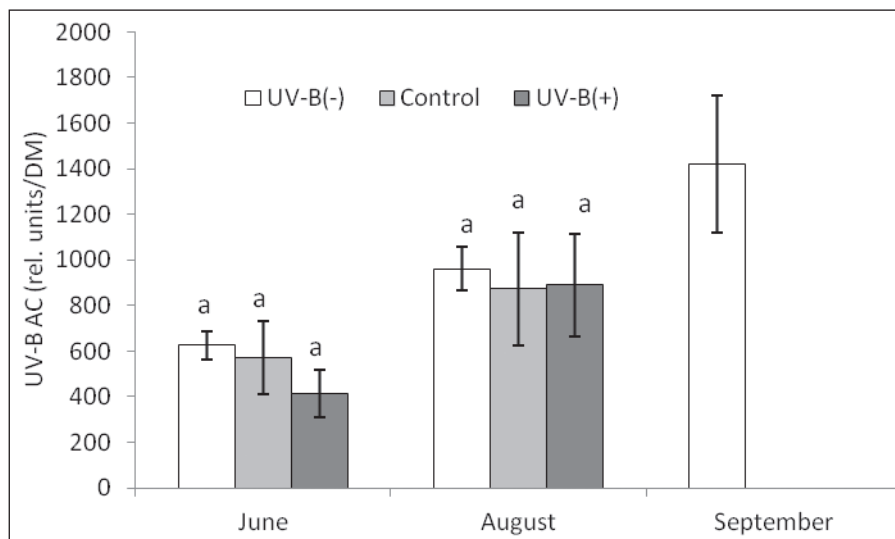


Figure 1. Production of UV-B absorbing compounds (UV-B AC) in leaves of *Tropaeolum majus* exposed to different UV-B treatments. Results are mean values \pm SD (standard deviation), $n = 5$. UV-B(-) – reduced level of UV-B radiation, control – ambient radiation, and UV-B(+) – elevated UV-B radiation. Different letters indicate significant difference at $p \leq 0.05$ confidence level among values within each sampling.

Figure 2. Production of UV-B absorbing compounds (UV-B AC) in flowers of *Tropaeolum majus* exposed to different UV-B treatments. Results are mean values \pm SD (standard deviation), $n = 5$. UV-B(-) – reduced level of UV-B radiation, control – ambient radiation, and UV-B(+) – elevated UV-B radiation. Letters indicate that there was no significant difference ($p \leq 0.05$) among values within each sampling.



Saile-Mark and Tevini (50) report that enhanced UV-B radiation delays the start of flowering in the majority of studied cultivars of *Phaseolus vulgaris*. The possible cause is the effect of UV-B radiation on the biosynthesis of gibberellins.

The efficiency and stability of PSII, the major component of the photosynthetic apparatus, was monitored during the vegetation season in terms of F_v/F_m . Alteration in F_v/F_m implies changes in photochemical conversion efficiency of PSII and, therefore, possible photoinhibition of photosynthesis. Under non-stressed conditions, F_v/F_m is almost constant (from 0.80 to 0.86) (51). In our experiment, potential and effective quantum yields of PSII did not differ between treated and untreated plants, with the exception of a negative effect of UV-B radiation on effective quantum yield of PSII in peak season (August). Measured values of F_v/F_m ranged from 0.75 to 0.83 and of $\Delta F/F_m'$ from 0.37 to 0.50 (Tab. 2). Close values of the potential photochemical efficiency to the theoretical maximum indicated an undamaged antenna complex (26). Even though the effective yield was lower than the potential one (Tab. 2), the high values of potential yield, indicated reversible inactivation rather than damage to the reaction centre. UV-B radiation has been reported to hit several specific targets on the electron transport side of the PSII reaction centre, resulting in inefficient use of energy (52). Lingakumar et al. (53) stated that UV-B exclusion promoted PSII activity of *Vigna unguiculata*. On the other hand it has been reported that solar UV-B filtering did not cause any change in the photochemical efficiency of PSII in common buckwheat (54), and in *Deschampsia antarctica* and *Colobanthus quitensis* (55), indicating that the photosynthetic apparatus was not damaged.

Photosynthesis was unaffected by UV-B radiation (Tab. 2). In contrast, Mohammed and Tarpley (30) showed that, for most rice cultivars, exposure to enhanced

UV-B radiation resulted in decreased photosynthetic rate compared to plants grown in a UV-B-free environment.

Transpiration rate increased under UV-B radiation treatment at the beginning and peak of the season (Tab. 2). These results contrast with those for wheat, pea and soybean exposed to UV-B (56). The effect of UV-B radiation on stomatal conductivity may be due to a change in stomata functioning (57).

During experiment transpiration rate in general decreased from June to September, while photosynthesis increased. Water use efficiency (WUE) increased throughout the season (Fig. 3) and was independent of UV-B radiation which is in apparent contrast to results on wheat cultivars (*Triticum aestivum*) (58). In that study authors evidence that UV-B radiation decreases WUE in comparison to the control. However, some soybean cultivars may respond to increased levels of UV-B by increasing WUE and this response could be manifested through changes in stomatal development and functioning (56).

UV-B did not affect specific leaf area or the length of the internodes or petiole in accord with results reported in the study on the St John's Wort (36). Measured values of specific leaf area in *T. majus* ranged from 0.29 to 0.33, length of the internodes from 1 to 1.5 cm, and length of the petiole from 7.8 to 9.6 cm. However, UV-B caused stunting of plant stems in both common and Tartary buckwheat species (37). Ballaré et al. (59) concluded that the inhibition of stem elongation in various plants induced by UV-B is either a direct consequence of damage to proteins or is induced by cellular signals resulting from DNA damage or oxidative stress.

There is a strong correlation between the accumulation of phenolic compounds and UV-B tolerance. The level of DNA damage caused by UV-B radiation was lower in plants with accumulated phenolic substances, as in the

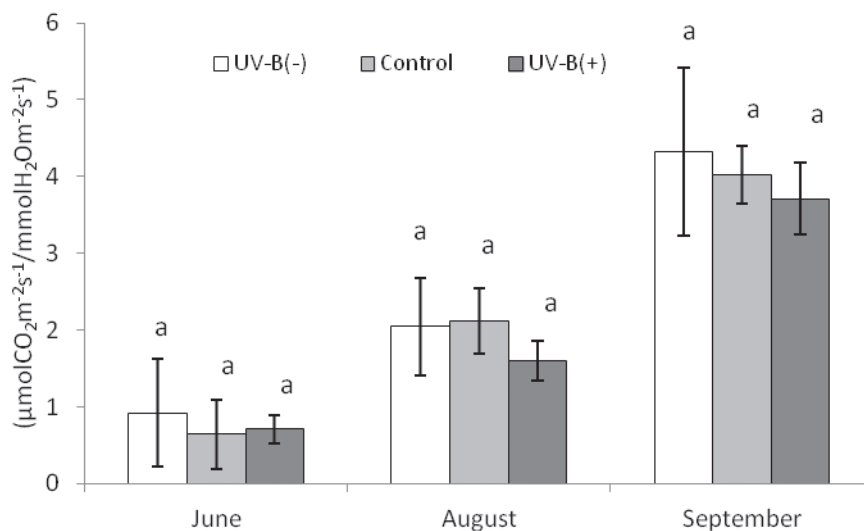


Figure 3. Water use efficiency in *Tropaeolum majus* grown under different UV-B treatment. Results are mean values \pm SD (standard deviation), $n = 5$. UV-B(-) – reduced level of UV-B radiation, control – ambient radiation, and UV-B(+) – elevated UV-B radiation. Letters indicate that there was no significant difference ($p \leq 0.05$) among values within each sampling.

leaves of barley (60). It is clear that in *T. majus*, the protection caused by accumulation of UV absorbing compounds was effective, since photosynthesis, photochemical efficiency, length of internodes and petiole did not differ at three levels of UV-B radiation. The resistance of *T. majus* to UV-B radiation is consistent with the fact that it originates from high altitude areas. The absence of apparent photosynthetic and growth damage in the present study is not uncommon and parallels results from several recent studies on other plant species in which direct damage to plants by UV-B was not found (58).

Searles et al. (61) summarized 450 reports from 62 papers regarding the effect of UV-B radiation on plants in field-based studies. They also found out that effects of UV-B were most apparent for the case of UV-B-absorbing compounds with an average increase of approximately 10% across all studies when comparing the ambient solar UV-B control to the treatment. Plant height and leaf mass per area showed little or no response to enhanced UV-B. Chlorophyll fluorescence and the concentration of photosynthetic pigments were also not affected.

Flint et al. (62) reported that both UV-B-exclusion and UV-B-supplementation studies on ecosystems have rarely been conducted simultaneously. Such experiments were initiated in a dune grassland ecosystem in The Netherlands (52.5 °N) (63). Results show that in the UV exclusion experiment, near-ambient UV-B caused some inhibition of plant growth, but lamp UV-B-supplementation had minimal additional effect.

CONCLUSIONS

Biologically effective UV-B doses ($UV-B_{BE}$) corresponding to 17% reduction of the ozone layer had no effect on specific leaf area, the length of internodes or

petiola, photosynthesis or potential photochemical efficiency of PSII. Such high resistance is probably the consequence of successful acclimation to UV-B radiation in the natural environment. UV-B treated specimens of *T. majus* possessed enhanced amounts of total phenolic compounds, which are important for utilisation of *T. majus* herbs for human health. Enhanced UV-B treatment affected flowering of *T. majus* at the end of the growing season, which may have an important negative implication for success of this species in elevated UV-B radiation environments. The flowers of *T. majus* are the source of active substances for the pharmaceutical industry, therefore these results also have economic significance.

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