Growth and production of buckwheat (*Fagopyrum esculentum*) treated with reduced, ambient, and enhanced UV-B radiation

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Abstract

The effect of enhanced UV-B radiation on buckwheat (*Fagopyrum esculentum* Moench. variety ‘Darja’), an important high elevation crop, was studied in order to estimate its vulnerability in changing UV-B environment. Plants were grown in outdoor experiments from July to October under reduced and ambient UV-B levels, and an UV-B level simulating 17% ozone depletion in Ljubljana. During the development the following parameters were monitored: light saturated photosynthetic activity, transpiration, potential and effective photochemical efficiencies of photosystem II, the contents of photosynthetic pigments and methanol soluble UV-B absorbing compounds. At the end of the experiment, growth rate and production of seeds were estimated. In the following growth season the seeds collected from plants exposed to different UV-B treatments were tested for germination capacity. Total UV-B absorbing compounds during plant development were increased by UV-B radiation, photosynthetic pigments (chlorophyll *a* and *b* and carotenoids) decreased. Photosynthetic rate was lowered in an early stage of development. UV-B treatment resulted in the increase in the transpiration rate and consequently the decrease in water use efficiency (WUE). The disturbances in water economy and in photosynthesis affected the reproduction potential negatively; the production of seeds in plants cultivated under ambient and enhanced UV-B was 57 and 39% of the production of specimens treated with reduced UV-B, respectively. The germination of seeds collected from treated plants revealed on average about 95% success, independently of the treatment, but the time needed for germination was the shortest for seeds developed under enhanced UV-B level treatment. Enhanced UV-B radiation affected water relations and production of buckwheat, but not the potential of seeds for germination.

Keywords: *Fagopyrum esculentum*; Crop; Ultraviolet-B radiation; Water use efficiency; Seed production

1. Introduction

The amount of UV-B radiation reaching the Earth surface depends on a variety of conditions, among them, altitude present a significant factor [1]. Many investigations concerning the influence of UV-B radiation on different plants species were carried out during the last 25 years, but relevant studies on agricultural plants conducted in outdoor environments are scarce and the outcomes are variable [2]. Buckwheat is an important agricultural plant thriving at higher altitudes which are not suitable for cereals and rice. The history of cultivating buckwheat goes back to ancient times [3] and today this crop is still an important food source. It is an annual plant, with a short vegetation period and it does not possess a special strategy of investing for safety as do biannual and perennial plants [4]. Buckwheat must make full use of a short growing period. Any stress puts additional demands on the plant for establishment of protective and repair mechanisms and could result in lower yield and reproduction potential. As an agricultural plant, buckwheat has been subjected to intensive breeding in order to improve the production of grains [5]. Developing grains present a significant attraction centre for photosynthates, consequently the production of secondary substances which is also an energy demanding process could be affected [6]. It is possible that therefore the potential of buckwheat for coping with different stresses is also lowered.

In the present study we simulated reduced and ambient level UV-B radiation and an increased level corresponding to 17% ozone depletion, in order to estimate the effects of UV-B radiation on the production of UV-B absorbing compounds, photosynthetic pigments, efficiency of light use, photosynthetic activity and transpiration rate and to find out whether the enhanced level presents a threat to development and production of seeds in buckwheat.

2. Material and methods

2.1. *Plant material and growth conditions*

Buckwheat (*Fagopyrum esculentum* Moench. variety ('Darja')) seeds were sown in a sandy soil in pots (30–40 seeds per pot, the size of the pot was 50350319 cm) on an outdoor research plot (Botanical garden, University of Ljubljana, Ljubljana, Slovenia, 320 m above sea level, 468359 N, 148559 E) at the end of July in 1999. Plants were watered regularly. The samples for the analyses of plants growing under each treatment were chosen randomly out of 100 specimens.

The UV-B supplement system was designed as described in the literature [7]. Three different treatments were applied: simulation of 17% ozone depletion (20 cm above ground level) (UV-B (+)) using Q-Panel UV-B 313 lamps (Cleveland, OH, USA), filtered with cellulose diacetate filters, which cut out UV-C range (radiation of wavelength lower than 280 nm), reduced level of UV-B radiation (UV-B (–)) using Mylar foils which cut the wavelengths below about 320 nm [8] and ambient radiation with Q-Panel UV-B 313 lamps filtered with Mylar foil, to correct for effects of the UV-A radiation (control). In (UV-B (–)) level we measured on average a 40% UV-B radiation dose reduction. The (UV-B (+)) and control systems consisted of six Q-Panel UV-B 313 fixed in an aluminium frame (2.031.2 m), while for (UV-B (–)) treatment Mylar foil was fixed in the frame of the same size positioned 80 cm above the plants. The systems were timer controlled. The doses simulating 17% ozone depletion were calculated and adjusted weekly according to the programs made by Björn and Murphy [9] using the generalised plant action spectrum [10]. Air temperature was recorded continuously (Data Logger, LI-COR, Lincoln, NE, USA) at the site (Fig. 1). Ambient UV-B, UV-A and PAR were monitored by a three-channel dosimeter (ELDONET) belonging to the European Light Dosimeter Network (Fig. 2).

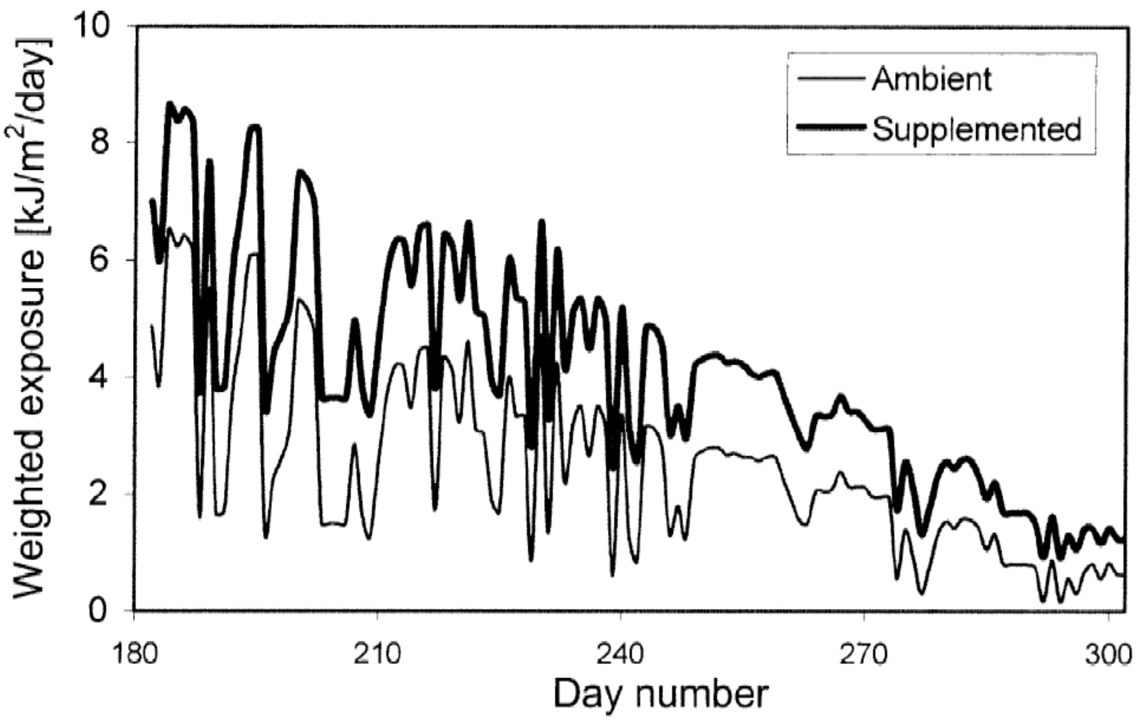

Fig. 1. Photosynthetically active radiation (PAR) and average daily air temperatures during the experiment with buckwheat (*Fagopyrum esculentum*).

2.2. *Photosynthetic pigments and UV-absorbing substances*

Total chlorophyll content, chlorophyll *a* /*b* ratio were determined as reported by Jeffrey and Humphrey [11] and carotenoid content according to Strickland and Parsons [12]. Plant material was weighed, homogenised in extraction solution (90% (v/v) acetone) and centrifuged (10 000 rev./min, 4^{o}C, 4 min). Pigment content was calculated per sample dry matter based on absorbency at 480, 630, 647, 664 and 750 nm measured with a UV/ VIS SpectrometerUV-B absorbing substances were extracted with metha-nol:distilled water:HCl579:20:1 (v/v/v) from fresh homogenised plant material [10]. The samples were centrifuged and the absorbency of supernatants measured and integrated over the range 280–320 nm. The extinction values were integrated and corrected for the weight of the sample (approximately 0.1 g DW).

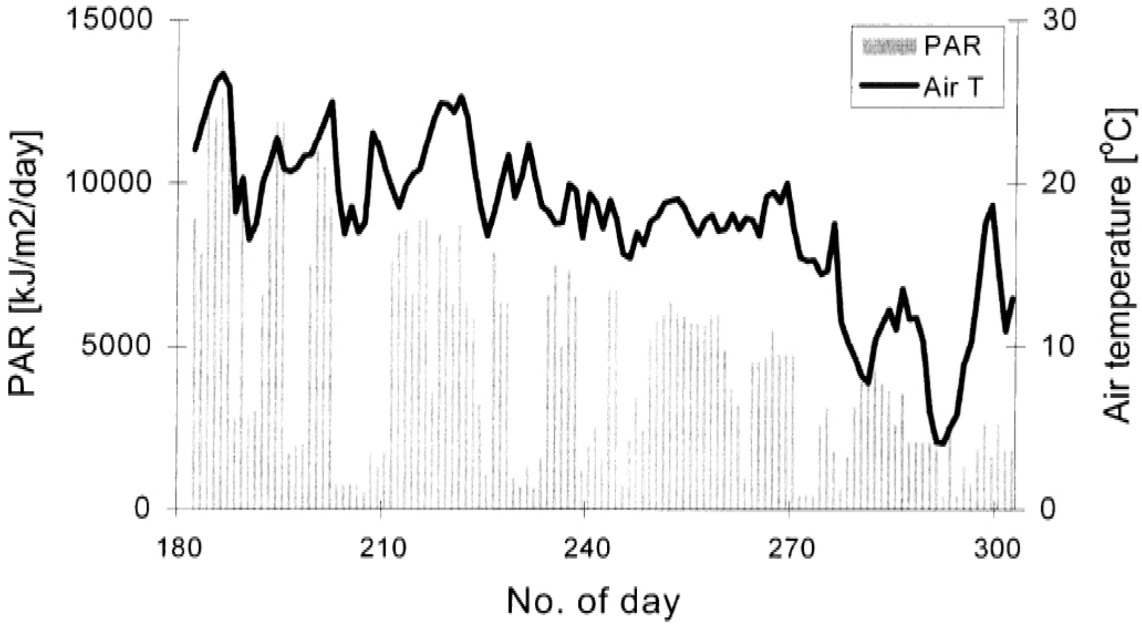

Fig. 2. Natural and supplemented UV-BBE radiation in buckwheat experiment measured 20 cm above ground level.

2.3. *Net photosynthesis*, *transpiration rate and chloro*Measurements of light-saturated net photosynthesis and transpiration rate were performed with a portable infrared gas analyser (ADC, LCA 4, UK). The measurements were carried out on clear days at noon by sun time (PPFD more than 1100 mmol m^{-2} s^{-1}) at ambient temperature and CO_2 concentration conditions. Water use efficiency (WUE) was calculated as the ratio between net photosynthesis and transpiration rates. Photochemical efficiency was estimated from variable chlorophyll *a* fluorescence of photosystem II (PS II) using a modulated fluorometer (OS-500; OPTI-SCIENCES, Tyngsboro, MA, USA). Measurements were carried out on clear days around noon. After 10 min of darkness, provided by dark-adaptation clips, fluorescence was excited with a saturating beam of white light (PPFD = 8000 mmol $m^{-2}s^{-1}$, 0.8 s) to determine the maximum (F_m) and the minimum (F_o) fluorescence of the dark-adapted sample. Optimal quantum yield was expressed as $F_v / F_m = (F_m - F_o)/F_m$. Effective quantum yield was determined by providing a saturating pulse of white light to the leaf surface using the standard 60° angle clip. The Y coefficient was defined as $F'_v / F'_m = (F'_m - F'_o)/F'_m$, where F'_m represents maximal and F'_o minimal fluorescence of an illuminated sample.

2.4. *Growth analysis and germination experiment*

Growth analysis was performed after termination of the experiment in the beginning of October. The length of the internodes, the height of plants and biomass partitioning into different organs were examined. Plant material was oven dried at 105 8C for 24 h. Prior to drying, the underground parts were washed thoroughly to remove different particles.

The germination experiment was carried out following the conditions prescribed by ISTA [13], at temperatures from 20 to 30 8C, with no pretreatment. Seeds collected from the three UV-B treatments in the previous year were germinated between two moist filter papers in polyethylene containers. The germinating seeds were counted every day. A seed was considered germinated when radicula appeared. Three parallels with 100 seeds were monitored for each treatment.

2.5. *Statistical analysis*

The statistical analysis was carried out using the *F*-test and Student's *t*-test (Excel Data Analysis). The significance of the differences among treatments is presented as a comparison between UV-B (+) and UV-B (–) treatment (*) and between UV-B (+) and control treatment as (+), (P<0.05).

3. Results and discussion

Enhanced UV-B radiation treatment affected growth and production of buckwheat. The defence strategy against enhanced UV-B radiation in buckwheat is a consequence of the short vegetation period and the fact that it is annual plant. Even though previous research revealed that the accumulation effect occurs mainly in perennial plants [14], the production of UV-B absorbing compounds in buckwheat did not respond to the seasonal UV-B radiation changes but the accumulation of the compounds occurred in assimilation tissue during plant development. UV-B absorbing compounds belong mainly to flavonoids, and also contribute to the filtering of harmful rays and mitigate against the effects of free radicals [15]. The total amounts in plants cultivated under ambient and enhanced UV-B levels were higher compared to those grown under reduced UV-B (Fig. 3).

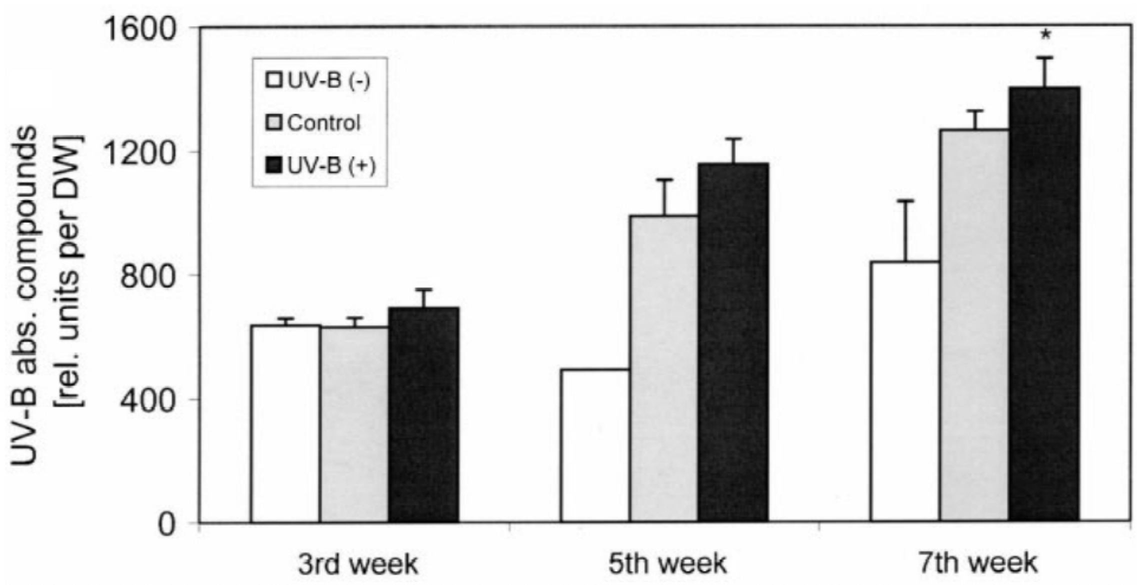

Fig. 3. The production of UV-B absorbing compounds per dry weight (DW) in buckwheat plants growing under different treatments during the growth period. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control as (+).

The absorption spectra of methanol extracts of treated leaves in the UV range revealed an evident increase also in UV-A range (Fig. 4). In experiments with *Brassica rapa* where plants were treated with UV-B radiation corresponding to 16% ozone reduction no significant increase of production of UV-B absorbing compounds was found [16]; however, double doses exerted an effect. Research on the influence of enhanced UV-B on *Brassica napus* revealed an increase in two main groups of flavonoids kampferol and quercetin glycosids [17].

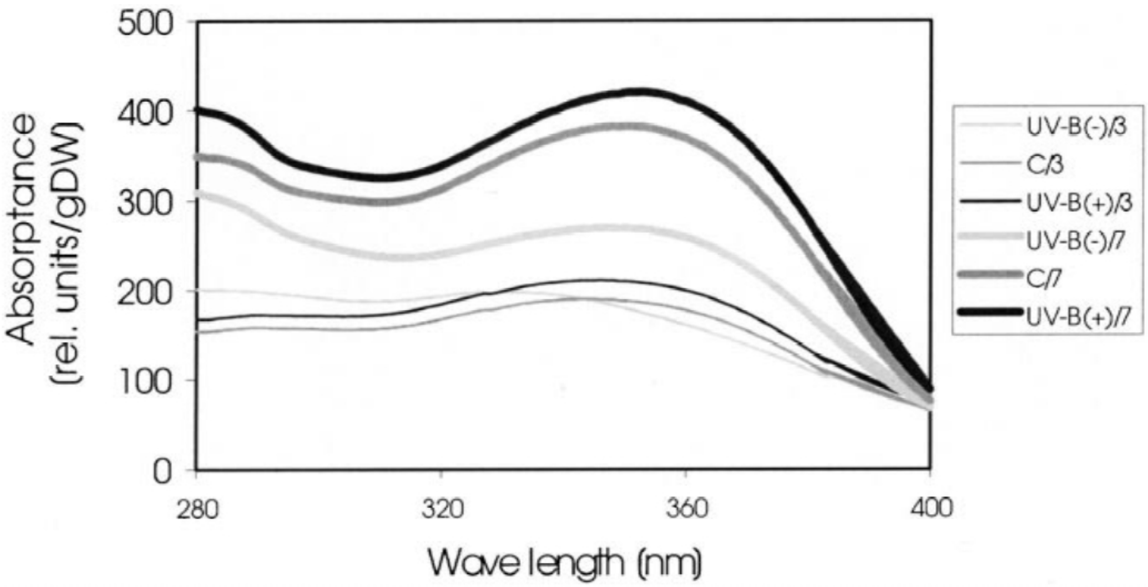

Fig. 4. The average absorption spectra of acidified methanol extract of leaves (corrected for dry weight of samples) from different treatments in the beginning (3rd week) and at the end (7th week) of the vegetative period (n = 4–5).

The poor production of UV-B absorbing compounds during early development of buckwheat could possibly be one of the reasons for a disturbance at the biochemical and physiological level. Chlorophyll *a* and *b* and carotenoid contents were slightly affected but the *a* /*b* ratio remained unchanged under the different treatments (Fig. 5). The literature reports on UV-B influences on photosynthetic pigments [18–20]. Sunflower and corn showed little effect of UV-B on chlorophyll content [21], as did *Brassica rapa* [16] and *Phaseolus vulgaris* [22]. In buckwheat, potential and actual photochemical efficiencies of PS II were not affected by enhanced UV-B radiation (Fig. 6). This is in contrast to UV-B treated *Pisum sativum* in which a significant disturbance in light utilisation was detected [23]. In our experiment net photosynthesis had been disturbed in an early phase of development, probably due to the low amount of UV absorbing pigments. Photosynthesis was significantly lower in plants treated with enhanced and ambient in comparison to reduced UV-B (Fig. 7). The increased production of UV-B absorbing compounds at later stages afforded some protection for potential targets in the mesophyll and therefore photosynthetic activity was less affected. Previous studies of photosynthetic response revealed that buckwheat thrives over a wide temperature range but is sensitive to frost at the end of the vegetative season and this could additionally affect the production [24]. But it was not the case in our study (Fig. 1). The decrease of net photosynthesis due to enhanced UV-B was detected also in some other agricultural plants, i.e. in soybean and pea [20,23] but not in sunflower, corn and bean [21,22]. It seems that the significant increase of transpired water in young UV-B treated buckwheat plants influenced their further development. Changes in photosynthetic as well as transpiration rates resulted in a drastic decrease in WUE, which was most pronounced at an early stage of development (Fig. 7). The possible reason could be disturbances in the functioning of stomata. Disturbances in water economy and water deficiency are factors leading to reduced plant production [4]. Studies of UV-B effects on water relations in corn and sunflower showed no disturbance. These results are also supported by experiments with wild species *Calamagrostis epigeos* and *Silene vulgaris* [25].

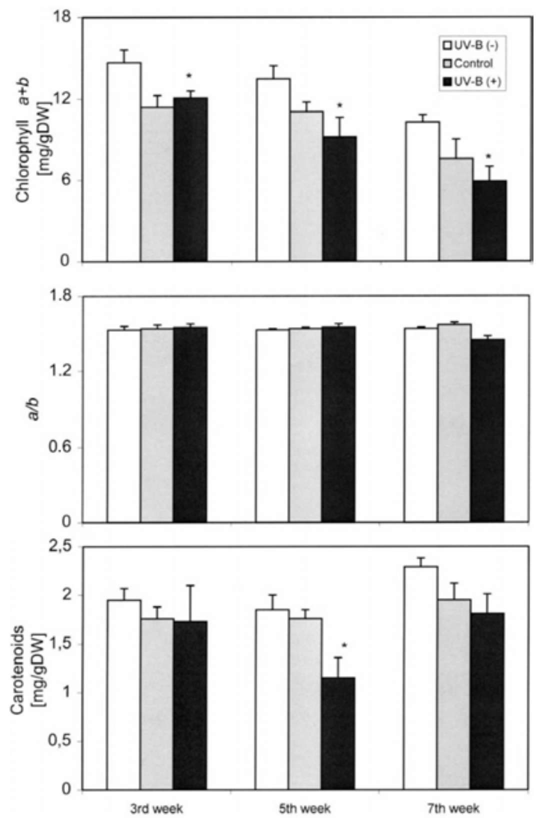

Fig. 5. The changes in chlorophylls *a* and *b* contents, chlorophyll *a* /*b* ratio and carotenoid content in buckwheat plants growing under different treatments during the growth period. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) andbetween UV-B (+) and control treatment as (+), (P<0.05, n = 4–6).

Specific leaf weight in buckwheat treated with enhanced UV-B was significantly increased only in comparison to the plants treated with reduced, but not ambient UV-B (Fig. 8). Similar results were obtained with *Brassica rapa* [16] and *Silene vulgaris* [26]. Researchers using higher doses to treat sunflower and corn, report an increase in leaf thickness [21]. Treatment of eight varieties of beans [27] showed that the differences in the response are not interspecific only, but intraspecific as well. Plant stunting is another common UV-B induced morphogenetic phenomenon, which occurs primarily due to shorter internodes and reduction of internode numbers [28]. In the case of buckwheat no significant differences were determined in the number and length of the internodes, even though plant height was decreasing with UV-B dose (Fig. 9).

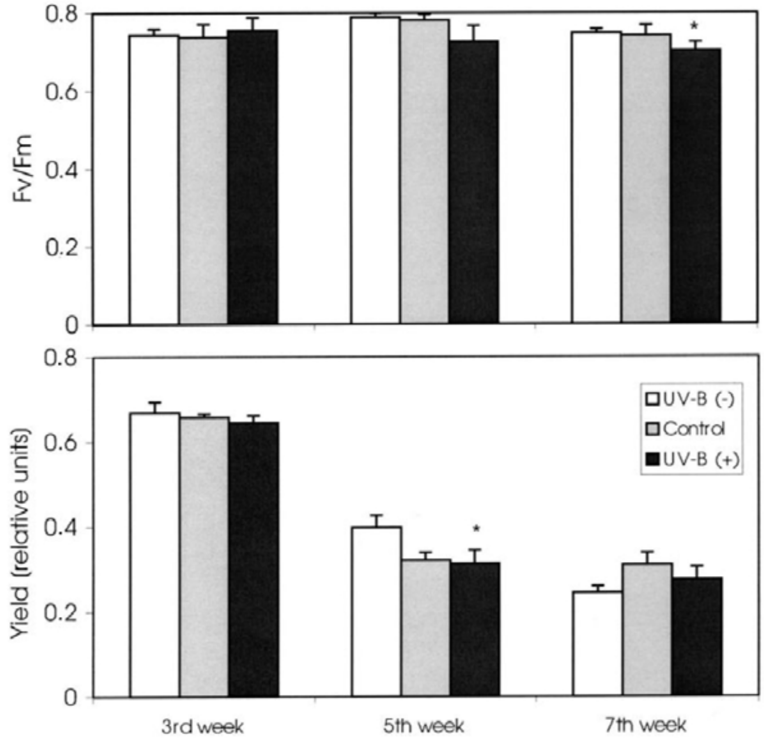

Fig. 6. Potential (upper graph) and actual (lower graph) photochemical efficiency of PS II in buckwheat plants growing under different treatments during the growth period. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control treatment as (+), (P<0.05, n = 8).

Disturbed physiological processes, above all WUE and costly synthesis of UV-B absorbing compounds in treated buckwheat plants resulted in lower biomass production. The ratio among biomass allocation to roots, stems, leaves and seeds was similar in all treatments, but total biomass was significantly higher in plants treated with reduced level of UV-B (Fig. 10). Reduction of biomass is not necessarily connected to disturbances in photosynthesis and photochemical efficiency of PS II, but it is also a consequence of carbohydrate partitioning [29]. Reduction of biomass was shown also in the greenhouse experiments with bean, where the dry weight of aboveground parts and roots were reduced by 24% and 14% at the highest UV-B radiation level (12 kJ m^{-2} day^{-1}) [30].

The most sensitive time for developing plants is the transition from vegetative to reproductive phase [31]. Successful reproduction is an important aspect in agricultural plants. In buckwheat this is the production of seeds (Fig. 11). The seed production for reduced, ambient and enhanced UV-B was 33, 19 and 13 seeds per plant, respectively, the average weight of the single seed being 0.88, 0.45 and 0.32 g. The experiments with lowland and highland population of *Silene vulgaris* reveals a decrease of seed number in the former and an increase in the latter [32]. The impact of enhanced UV-B radiation on seed production of soybean, rice, pea and mustard were reviewed by Calldwell and co-workers [13]. Literature data are contrasting, but the studies reporting negative effects are more frequent.

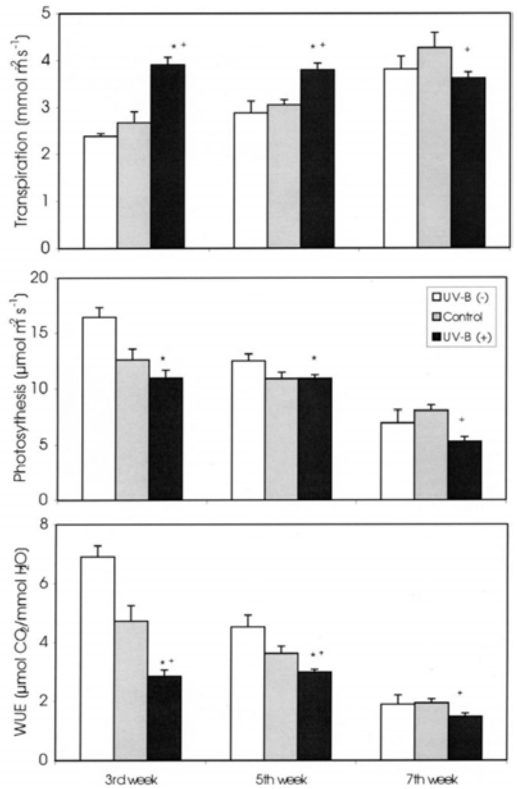

Fig. 7. Transpiration rate, net photosynthetic activity and water use efficiency in buckwheat plants growing under different treatments during the growth period. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control treatment as (+), (P<0.05, n = 7–15).Fig. 9. Length of internodes in buckwheat plants growing under different treatments at the end of the growth season (n = 4–5).

Germination of seeds ripened under the UV-B treatment experiment in the following vegetative season exhibited onaverage about 95% success rate, independent of treatment (Fig. 12). Difference was observed in the time needed for germination, which was shortest for UV-B treated plants, possibly as a consequence of poorly developed testa.

It is concluded that *F. ecsulentum* is sensitive to UV-B stress. As an annual plant it should use a short vegetation period in which conditions are favorable for growth, flowering and setting of seeds [4]. This strategy is successful under favourable environmental conditions. When the conditions become stressful, more energy is directed to mitigate the stress events, this leads to reduced photosynthetic yield and reproductive potential. A high elevation environment with more severe climate, i.e. higher UV-B doses, lower temperatures, could have a direct effect on photosynthesis [24] and could also influence plant production. Disturbances in water economy which were detected in the present experiment where plants were watered regularly indicates also the increased vulnerability in the case of combined UV-B and water stress.

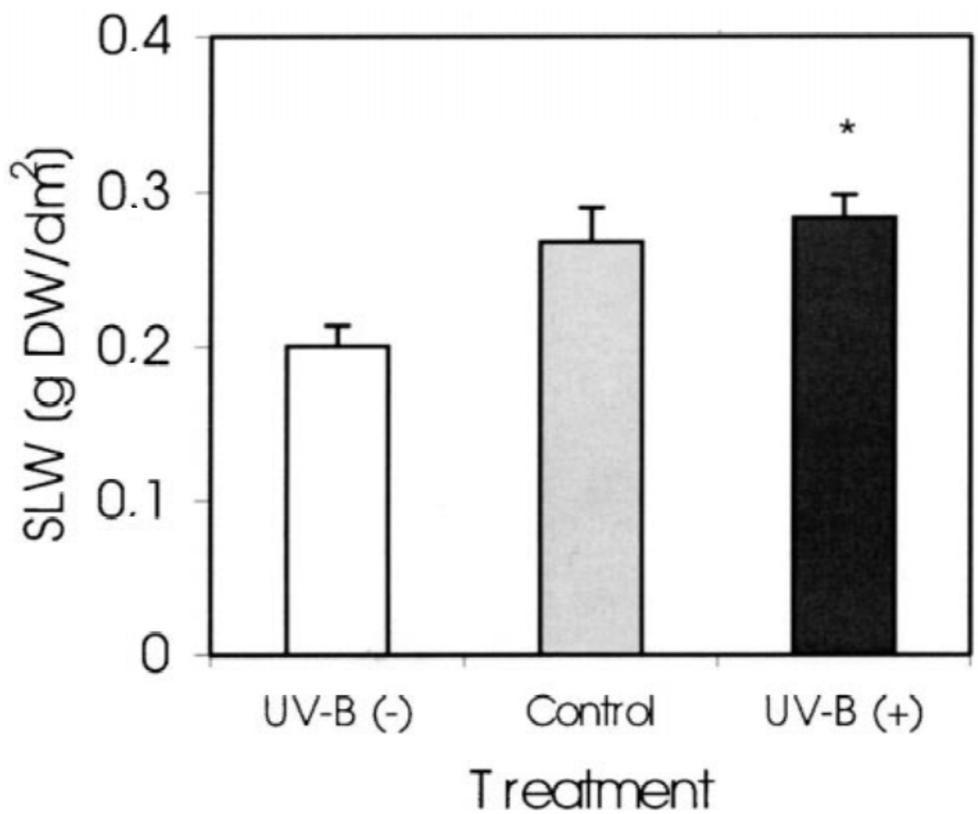

Fig. 8. Specific leaf weight in leaves of the buckwheat plants growing under different treatments at the end of the growth season. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control treatment as (+), ($P<0.05$, $n = 15$).

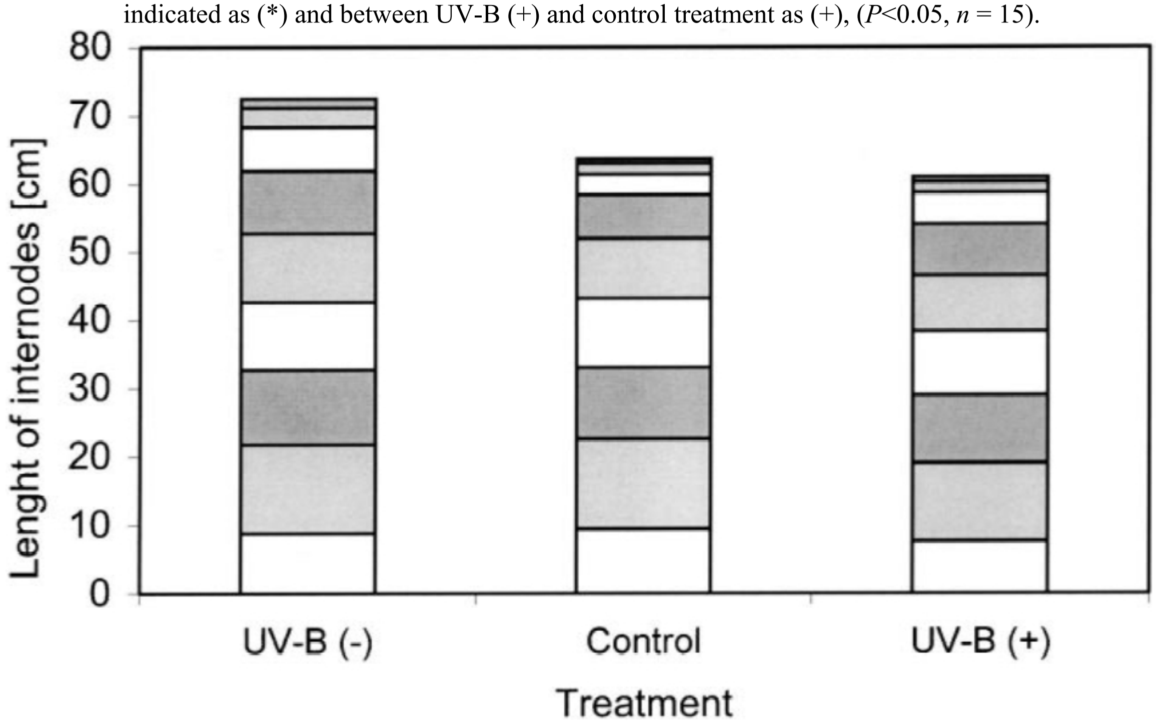

Fig. 9. Length of internodes in buckwheat plants growing under different treatments at the end of the growth season (n = 4–5).

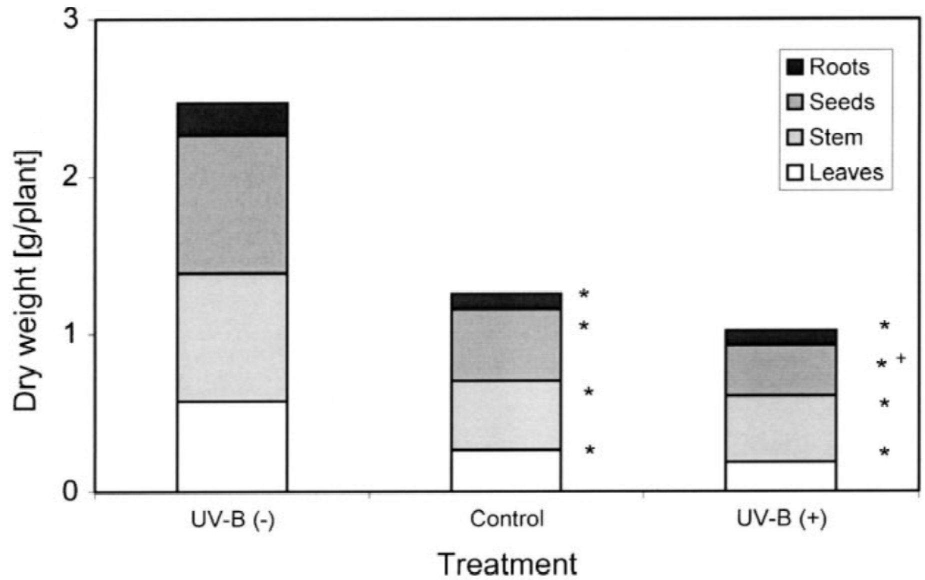

Fig. 10. Dry weight of different parts of the buckwheat plants growing under different treatments at the end of the growth season. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control treatment as (+), (P<0.05, n = 7).

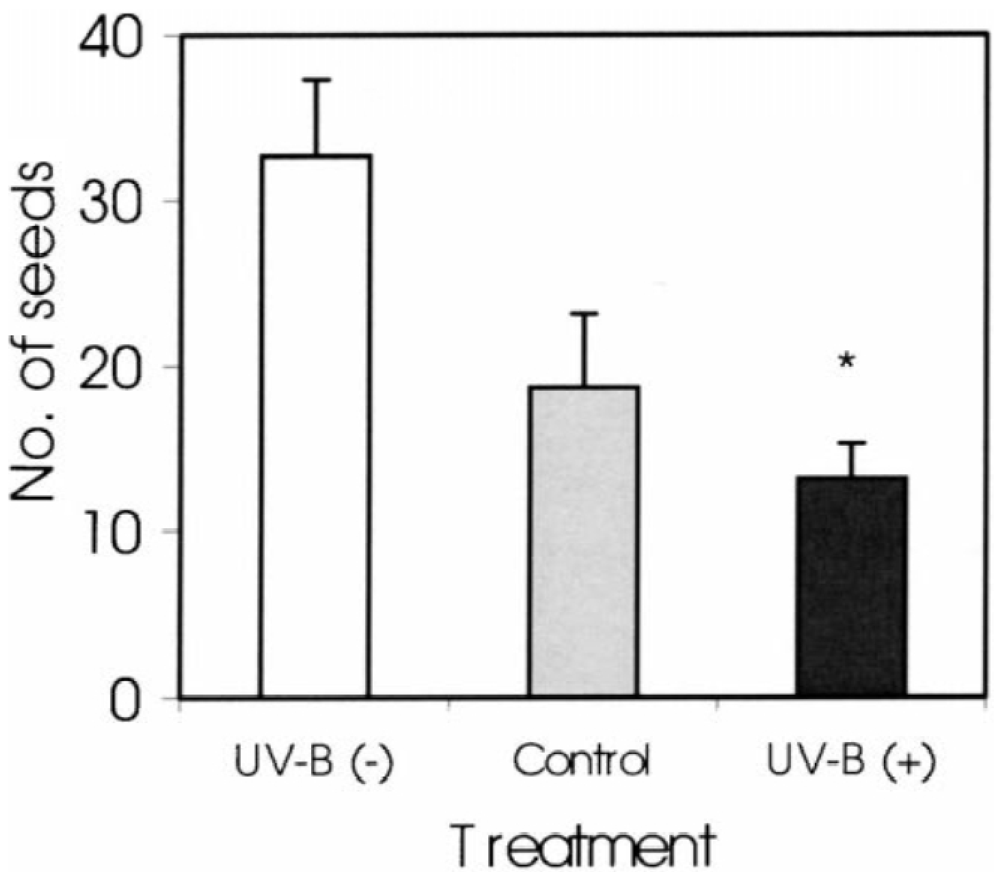

Fig. 11. Seed production per plant of the buckwheat plants growing under different treatments at the end of the growth season. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control treatment as (+), (P<0.05, n = 6).

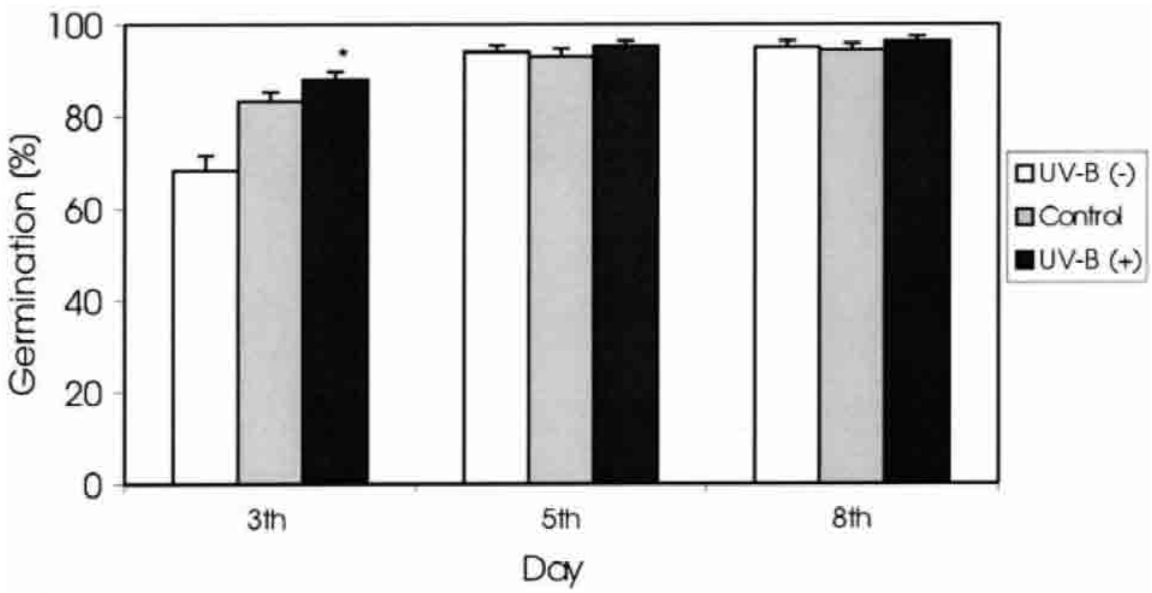

Fig. 12. Germination success of seeds of the buckwheat plants growing under different treatments in the following growth season. The significance of the difference between UV-B (+) and UV-B (–) treatment is indicated as (*) and between UV-B (+) and control treatment as (+), (*P*<0.05, *n* = 3).

Acknowledgements

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