

Combined effects of elevated UV-B radiation and the addition of selenium on common (*Fagopyrum esculentum* Moench) and tartary [*Fagopyrum tataricum* (L.) Gaertn.] buckwheat

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Abstract

The combined effects of UV-B irradiation and foliar treatment with selenium on two buckwheat species, common (*Fagopyrum esculentum* Moench) and tartary [*Fagopyrum tataricum* (L.) Gaertn.] buckwheat, that underwent different intensity of breeding, were examined. Plants grown outdoors under three levels of UV-B radiation were studied for 9 weeks, from sowing to ripening. At week 7 they were sprayed with solution containing 1 g(Se) m^{-3} that presumably mitigates UV-B stress. Morphological, physiological, and biochemical parameters of the plants were monitored. Elevated UV-B radiation, corresponding to a 17 % reduction of the ozone layer, induced synthesis of UV absorbing compounds. In both buckwheat species it also caused a reduction in amounts of chlorophyll *a* during the time of intensive growth, an effect, which was increased in tartary buckwheat in the presence of selenium. The respiratory potential, measured as terminal electron transport system activity, was lower in plants subjected to enhanced UV-B radiation during the time of intensive growth. The effective quantum yield of photosystem 2 was also reduced due to UV-B radiation in both buckwheat species and was mitigated by the addition of Se. Se treatment also mitigated the stunting effect of UV-B radiation and the lowering of biomass in common buckwheat.

Additional key words: biomass; chlorophyll; energy conversion; leaf area and thickness; plant height; UV-A.

Introduction

The intensity of UV-B radiation at the Earth's surface is increasing due to thinning of the ozone layer. Although only 0.3 % of UV-B radiation reaches the Earth's surface, it has an important effect on organisms (Jansen *et al.* 1998), including damage to DNA, alterations in transpiration, photosynthesis, respiration potential, growth, development, and morphology (Rozema *et al.* 1997, Björn 1999, Jansen 2002, Gaberščik *et al.* 2002a,b). Plants have evolved a variety of repair and protection mechanisms, depending on the specific nature of the different targets of UV-B radiation. These include increased biosynthesis of UV screening compounds, *i.e.* kaempferol, luteolin, rutin,

tricine, and apigenin which specifically absorb radiation in the range of 280–400 nm (Jansen *et al.* 1998, Olsson *et al.* 1999, Rozema *et al.* 2002). Scavenging of active oxygen and other radical species resulting from the absorption of UV-B photons can alleviate UV-damage. Recently it has been demonstrated that Se protects plants against UV-induced oxidative stress (Seppänen *et al.* 2003) and promotes the growth of plants subjected to high-energy radiation (Xue *et al.* 2001, Valkama *et al.* 2003). Ekelund and Danilov (2001) reported that Se might play a role in the repair mechanisms in *Euglena gracilis* during UV irradiation. There is also evidence

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Abbreviations: Chl – chlorophyll; DM – dry mass; F_0 – minimum fluorescence in the dark adapted leaf (all reaction centres are open); F_m – maximum fluorescence in the dark adapted leaf (all reaction centres are closed); F_v – variable fluorescence ($F_v = F_m - F_0$); F_m' – maximum fluorescence of an irradiated sample; F_0' – minimal fluorescence of an irradiated sample; F_v/F_m – potential quantum yield of PS2; INT – iodo-nitro-tetrazolium-chloride; PPFD – photosynthetic photon flux density; PS – photosystem; Se – selenium; UV – ultraviolet.

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of Se involvement in anti-oxidative processes in potatoes (Seppänen *et al.* 2003), which could be important, since UV-B induces the production of free radicals and toxic oxygen species (Olsson 1999). Plant species and crop cultivars vary in their sensitivity to UV-B radiation (Cybulski and Peterjohn 1999, Mazza *et al.* 2000, Gaberščik *et al.* 2002a, Caldwell *et al.* 2003). Common buckwheat is a result of intensive breeding while tartary buckwheat is a domestic variety (Bonafaccia *et al.* 2003).

Materials and methods

Seeds of common buckwheat (*Fagopyrum esculentum* Moench) cv. Darja and tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.], domestic population from Luxembourg (originating from south-western China, 2 500–3 000 m a.s.l.), were sown outdoors in sandy soil in pots (50×50×19 cm) (Botanical Garden, University of Ljubljana: altitude 320 m, 46°35'N, 14°55'E) at the end of May. A “step wave” UV-B supplement system was designed as described by Björn and Teramura (1993). Elevated UV-B radiation, simulating 17 % ozone depletion, was produced using *Q-Panel UV-B 313* lamps filtered with cellulose diacetate filters, which block the UV-C range (wavelengths lower than 280 nm). To correct for the effects of UV-A radiation, control plants were irradiated with *Q-Panel UV-B 313* lamps filtered with a *Mylar* foil that cuts out wavelengths lower than 318 nm. The systems were timer controlled. UV-B doses were calculated and adjusted weekly using the program of Björn and Murphy (1985) and based on the generalized plant action spectrum of Caldwell (1968). Plants were watered every day. All analyses were made on the middle part of the first fully developed leaf.

Foliar treatment was applied at the time of blossoming, just after week five. Plants were sprayed with distilled water containing 1 g(Se) m⁻³ in the form of Na-selenate to wet the whole leaf area. Control plants were sprayed with distilled water, with no detectable amounts of Se.

Biochemical and physiological analyses were made in weeks 5, 7, and 9 of the buckwheat growth. Morphological analyses (leaf area, leaf thickness, plant height) and biomass determination were performed after termination of the experiment.

Contents of photosynthetic pigments were determined as described by Jeffrey and Humphrey (1975) using a UV/VIS Spectrometer System (*Lambda 12*, Perkin-Elmer, Norwalk, CT, USA).

Determination of total UV absorbing compounds: The basic procedure for extracting total UV absorbing compounds followed the method described by Mirecki and Teramura (1984). Fresh plants were homogenized in methanol/distilled water/HCl (v/v/v) = 79 : 20 : 1. After 20 min of incubation the samples were centrifuged in

Tartary buckwheat originates in higher altitudes at which UV-B radiation is more intense. Both species synthesise UV absorbing compounds (Bonafaccia *et al.* 2003, Fabjan *et al.* 2003). We hypothesised that the effects of enhanced UV-B radiation on common and tartary buckwheat might differ with respect to the intensity of breeding. We also presume that Se mitigates the UV-B stress.

a top refrigerated centrifuge (1 600×g, 10 °C, 10 min). The supernatants were scanned at intervals of 1 nm in the range of 280–400 nm for UV absorbing compounds. Absorbance of 280–320 nm for UV-B and from 320–400 nm for UV-A absorbing compounds was integrated and expressed per DM of the sample.

Photochemical efficiency of photosystem 2 (PS2) was measured using a fluorometer (*OS-500*, Opti-Sciences, Tyngsboro, MA, USA). The potential quantum yield was evaluated in terms of the ratio F_v/F_m . Measurements were made after 15 min of darkness, provided by dark-adaptation clips. F_0 was measured, followed by F_m , which was achieved by the application of a saturating beam of “white light” (PPFD = 8 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s). The effective quantum yield was determined by 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, under saturating irradiance (1 500–2 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at the prevailing ambient temperature. The yield coefficient was defined as $Y = (F_m' - F_0')/F_m'$, where F_m' is the maximum and F_0' the minimum fluorescence of an irradiated sample (Schreiber *et al.* 1995).

Terminal electron transport system activity: Respiratory potential was estimated by measuring the potential terminal electron transport system activity of mitochondria as reported by Packard (1971). Determination of terminal electron transport system activity is based on reduction of the artificial electron acceptor iodo-nitro-tetrazolium-chloride (INT) and on the spectrophotometric measurement of the rate of formazan production, which is directly related to the oxygen consumption of the tissue. Weighed plant material (0.01–0.02 g) was homogenized in ice-cold homogenization buffer in a mortar and with an ultrasound homogenizer (model 4710, 2–3 min; 40 W; Cole-Parmer, Vernon Hills, IL, USA) and centrifuged (8 500×g, 4 min, 0 °C) in a top refrigerated centrifuge (*2K15*, Sigma, Osterode, Germany). 0.5 cm³ of the supernatant was mixed in triplicate with 1.5 cm³ substrate solution, 0.5 cm³ INT-solution, and incubated at 20 °C for 40 min. After stopping the reaction with formaldehyde/phosphoric acid (1:1), the formazan absorption at 490 nm was determined. Terminal electron transport system activity was measured as the rate of INT reduction,

which was converted to the amount of oxygen (Kenner and Ahmed 1975) utilised per dry mass (DM) of leaves.

Morphological analyses: Cross sections of the leaves were analysed with the computer program *AnalySIS 3.0* (Soft Imaging System, Münster, Germany). Leaf area was measured with an area meter (*Delta-T Devices*,

Cambridge, England).

Statistical analyses: Samples for analysis were chosen randomly out of 100 plants grown under each treatment. The significance of effects of UV-B radiation, Se addition, and interaction was tested by multifactor ANOVA (*Statgraphics* version 4).

Results

Elevated UV-B radiation caused a reduction in amounts of Chl *a* in common and tartary buckwheat species in week 7, *i.e.* during the time of intensive growth of the plants ($p=0.0065$ and $p=0.0010$, respectively) (Table 2). In tartary buckwheat, Se also lowered the amount of Chl *a* significantly ($p=0.0147$). The ratio Chl *a/b* did not change on either UV-B or Se treatment up to week 7. At week 9, the ratio increased as the amount of Chl *b* decreased. A slight accumulation of UV-B absorbing compounds occurred in leaves during the phase of intensive growth and development (week 7) in both buckwheat species. UV-B radiation caused a significant increase ($p=0.0033$) of UV-B absorbing compounds in common buckwheat in week 7 and, in tartary buckwheat, in weeks 7 and 9 ($p=0.0003$ and $p=0.0411$) (Fig. 1). In plants treated with Se, UV-B radiation of tartary buckwheat did not result in any change of content of UV-B absorbing compounds in week 7 (statistical interaction, $p=0.0211$). UV-A absorbing compounds showed a pattern similar to that for UV-B absorbing compounds and multifactor ANOVA indicated a significant increase in content of UV-A absorbing compounds in both species following

enhanced UV-B irradiation (Fig. 1).

Enhanced UV-B irradiation and Se treatment, either singly or combined, had no effect on the F_v/F_m of PS2 in either species. The values were in average 0.79 ± 0.02 . Se treatment led to an increase in effective quantum yield of PS2 in week 7 ($p=0.0007$) in common buckwheat, and mitigated the decrease in effective quantum yield caused by UV-B (statistical interaction, $p=0.0483$) (Fig. 2); in week 9 only the effect of Se on the enhanced effective quantum yield was significant ($p=0.0063$). In tartary buckwheat, Se mitigated the reduction of effective quantum yield due to UV-B radiation in weeks 7 and 9 (statistical interactions $p=0.0246$ and 0.0062 , respectively) (Fig. 2).

Terminal electron transport system activity was highest at the beginning of the growth period (week 5). In weeks 5 and 7, UV-B radiation caused a decrease in terminal electron transport system activity in both buckwheat species. In week 9 there was no significant influence of either UV-B radiation or Se on terminal electron transport system activity (Fig. 2).

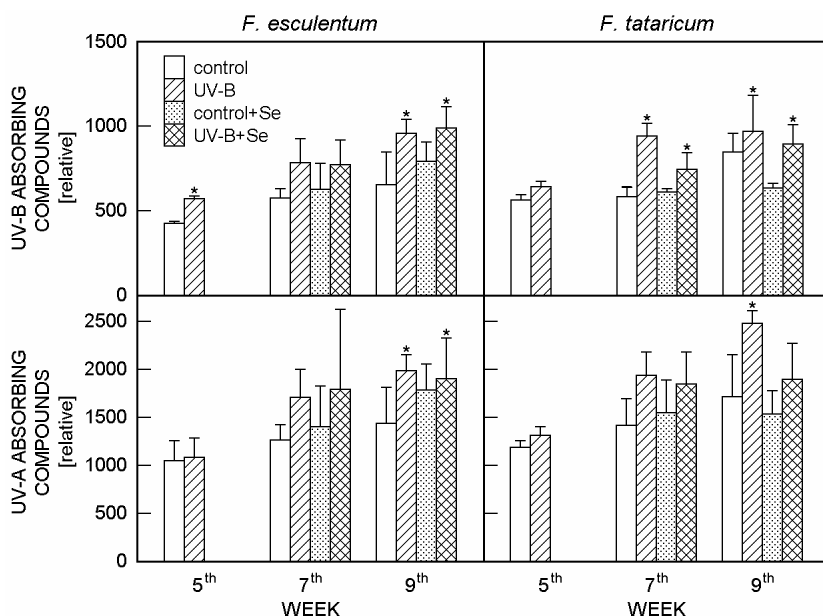


Fig. 1. The contents of UV-B (upper panels) and UV-A (lower panels) absorbing compounds per DM in common and tartary buckwheat grown under different UV-B and Se treatments ($n = 4$). The significance of differences in comparison to the control is indicated by * ($p < 0.05$).

Table 1. Morphological characteristics and biomass of common and tartary buckwheat grown under different UV-B and Se treatments at the end of the growing season. Means $\pm$ SD ($n = 8-10$). The significance of differences in comparison to the control is indicated by * ($p < 0.05$).

	Treatment	Leaf area [mm ²]	Leaf thickness [μ m]	Plant height [mm]	Biomass [g(DM)]
Common buckwheat	Control	790.38 $\pm$ 245.15	17.25 $\pm$ 0.89	820.2 $\pm$ 103.3	1.29 $\pm$ 0.18
	UV-B	375.88 $\pm$ 115.18*	20.50 $\pm$ 1.85*	585.9 $\pm$ 44.4*	0.63 $\pm$ 0.22*
	Control + Se	574.90 $\pm$ 194.64*	17.63 $\pm$ 3.11	636.4 $\pm$ 84.0*	0.86 $\pm$ 0.29*
	UV-B + Se	533.50 $\pm$ 168.37*	22.50 $\pm$ 2.45*	676.2 $\pm$ 55.3*	0.81 $\pm$ 0.33*
Tartary buckwheat	Control	263.67 $\pm$ 70.96	15.7 $\pm$ 1.7	512.7 $\pm$ 54.9	1.05 $\pm$ 0.50
	UV-B	99.56 $\pm$ 22.24*	22.8 $\pm$ 2.3*	419.7 $\pm$ 13.8*	0.55 $\pm$ 0.19
	Control + Se	226.40 $\pm$ 63.12	17.3 $\pm$ 1.5	543.2 $\pm$ 42.8	0.79 $\pm$ 0.47
	UV-B + Se	116.63 $\pm$ 25.04*	22.0 $\pm$ 2.2*	430.6 $\pm$ 28.6*	0.69 $\pm$ 0.24

Table 2. Chlorophyll (Chl) *a* and *b* contents [g kg⁻¹(DM)] in common and tartary buckwheat grown under different UV-B and Se treatments. Means $\pm$ SD ($n = 4$). The significance of differences in comparison to the control is indicated by * ($p < 0.05$).

	Treatment	Weeks 5		7		9	
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i>	Chl <i>b</i>
Common buckwheat	Control	7.28 $\pm$ 1.51	4.65 $\pm$ 0.74	7.23 $\pm$ 1.07	4.48 $\pm$ 0.80	6.65 $\pm$ 1.22	1.23 $\pm$ 0.29
	UV-B	5.91 $\pm$ 0.89	3.91 $\pm$ 0.54	5.15 $\pm$ 0.27*	3.19 $\pm$ 0.25*	5.17 $\pm$ 1.08	1.10 $\pm$ 0.10
	Control + Se			6.10 $\pm$ 1.00	3.49 $\pm$ 0.74*	3.93 $\pm$ 0.27	0.97 $\pm$ 0.08
	UV-B + Se			5.19 $\pm$ 0.89*	3.24 $\pm$ 0.50*	5.37 $\pm$ 1.14	1.33 $\pm$ 0.25
Tartary buckwheat	Control	6.18 $\pm$ 0.41	4.04 $\pm$ 0.44	9.31 $\pm$ 1.72	6.13 $\pm$ 1.06	6.87 $\pm$ 1.20	1.23 $\pm$ 0.33
	UV-B	6.92 $\pm$ 0.71	4.36 $\pm$ 0.21	7.32 $\pm$ 1.01*	4.69 $\pm$ 0.69*	5.55 $\pm$ 1.12	1.33 $\pm$ 0.24
	Control + Se			8.46 $\pm$ 0.48	5.27 $\pm$ 0.38*	5.30 $\pm$ 0.55	1.34 $\pm$ 0.15
	UV-B + Se			4.47 $\pm$ 0.23*	2.78 $\pm$ 0.13*	4.92 $\pm$ 1.10	1.17 $\pm$ 0.25

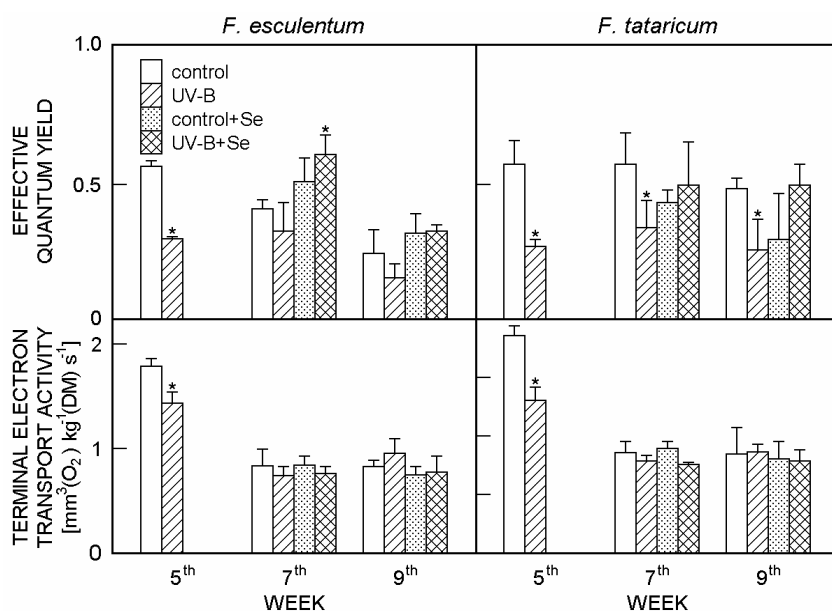


Fig. 2. Effective quantum yield of PS2 (*upper panels*) and terminal electron transport activity (*lower panels*) in common and tartary buckwheat grown under different UV-B and Se treatments ($n = 4$). UV-B irradiation and Se treatment, either singly or combined, had no effect on potential quantum yield of PS2 in either species. The values were in average 0.79 ± 0.02 . The significance of differences in comparison to the control is indicated by * ($p < 0.05$).

UV-B radiation caused stunting of plants, reduction of leaf area, and thicker leaves in both species (Table 1). In common buckwheat in the presence of Se, stunting was reduced and plants were taller (statistical interaction $p=0.0001$) (Table 1). In common buckwheat UV-B

Discussion

UV-B radiation reduced the amount of Chl *a* during the period of intensive growth of both species (Table 2) but this effect was not general. UV-B radiation causes either bleaching of photosynthetic pigments (Gaberšček *et al.* 2002b) or increase in their contents (Liu *et al.* 1995). Little effect of UV-B on Chl content was observed in sunflower and maize (Mark and Tevini 1997), *Brassica rapa* (Day and Demchik 1996), and *Phaseolus vulgaris* (Antonelli *et al.* 1997). Se decreased Chl *a* content in tartary buckwheat in week 7, independently of elevated levels of UV-B. The ratio of Chl *a/b* was unaffected by UV-B radiation, as known from the study of Day and Vogelmann (1995). At week 9, the ratio increased dramatically as the amount of Chl *b* decreased. The reason was degradation of Chl *b* due to senescence processes. Chl *b* is degraded after being converted to Chl *a* (Ito *et al.* 1996).

The contents of UV-A and UV-B absorbing compounds were higher under enhanced UV-B radiation in both species (Fig. 1), in line with our previous results on common buckwheat (Gaberšček *et al.* 2002b) and with those on other plants (Day and Vogelmann 1995, Day and Demchik 1996). The content of UV-B absorbing compounds was more variable in common buckwheat than in tartary buckwheat, possibly due to the fact that common buckwheat plants had larger leaf area (Table 1), resulting in a self-shading effect.

Neither enhanced UV-B radiation nor Se treatment affected the F_v/F_m of PS2 in either species as found in a similar study on barley and strawberry (Valkama *et al.* 2003). The values were in average 0.79 ± 0.02 , that was close to the theoretical maximum of 0.83 (Schreiber *et al.* 1995), indicating that the antenna complex was not damaged. F_v/F_m was reduced in *Medicago sativa* and *Brassica campestris* by UV-B radiation, but increased in *Brassica carinata* (Bornman and Vogelmann 1991). The addition of Se mitigated the negative effect of UV-B radiation on effective quantum yield of PS2 in both buckwheat species (Fig. 2). These results support the hypothesis that UV-B hits several targets, specifically on the electron transport side of the PS2 reaction centre, resulting in inefficient use of energy. The protective effect of Se is important because it improves photon harvesting and through this the availability of energy for plants.

Terminal electron transport system activity was highest during the period of intensive growth in week 5, due to the high metabolic demands for energy (Fig. 2), as

radiation induced a significant decrease of aboveground biomass ($p=0.0060$), which was ameliorated by Se ($p=0.0140$). In tartary buckwheat similar trends appeared which were, however, not statistically significant.

found for some other plants (Gaberšček *et al.* 2002a). UV-B radiation decreased terminal electron transport system activity in the peak of the season in both species, which is surprising, because of the claims of many authors that energy demands are increased during stress (Amthor 1995, Germ and Gaberšček 2003). For example, terminal electron transport system activity in *Ceratophyllum demersum* and green algae was significantly increased as a result of UV-B treatment (Gaberšček *et al.* 2002a, Germ *et al.* 2002).

UV-B radiation caused a reduction of the leaf size and thus diminished the area exposed to UV-B. It also increased the leaf thickness, presumably increasing the length of the path of UV-B rays between the leaf surface and, at least some of, the sensitive cellular sites (Table 1). UV-B caused stunting of plant stems in both species, an effect that was mitigated by Se treatment in common buckwheat (Table 1). Ballaré *et al.* (1996) concluded that inhibition of stem elongation in different 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. UV-B radiation caused decrease in aboveground dry mass in Se untreated plants only, indicating a potentially positive effect of Se, similar to the ameliorating effect of Se on stunting in common buckwheat (Table 1). A growth promoting effect of Se was observed in *Lolium perenne* (Hartikainen *et al.* 2000) and lettuce (Xue *et al.* 2001). Se may protect plants against UV-induced oxidative stress (Seppänen *et al.* 2003) and promote the growth of plants subjected to high-energy radiation (Valkama *et al.* 2003). Similarly, Hartikainen and Xue (1999) found that the addition of low concentration of Se promoted plant growth of UV-B irradiated plants. On the other hand, addition of Se did not change dry mass of strawberry under ambient radiation but, in combination with UV-B, decreased the dry mass of runners significantly (Valkama *et al.* 2003).

The found UV-B radiation effects only slightly differed between common and tartary buckwheat. Both species exhibited similar potential to cope UV-B stress since the production of UV-B and UV-A absorbing compounds reached the same level. Se treatment revealed a mitigating effect on effective quantum yield of PS2, as well as on stunting and biomass of common buckwheat plants. UV-B radiation and Se treatment obviously interfere with mechanisms of metabolism, growth, and development in both buckwheat species studied.

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