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Distribution of selenium and phenolics in buckwheat plants grown from seeds soaked in Se solution and under different levels of UV-B radiation

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ABSTRACT

Seeds of common buckwheat (*Fagopyrum esculentum*) were soaked in water, sodium selenate (5, 10 or 20 mg Se^{VI}/L), or sodium selenite (10 or 20 mg Se^{IV}/L) solutions. Plants grown from soaked seeds were exposed to reduced UV-B radiation, ambient, or enhanced UV-B. The mass fraction of selenium in leaves was much higher in plants obtained from seeds soaked with selenate (up to 185 ng/g) in comparison to selenite (up to 103 ng/g). In plants obtained from seeds soaked in water, regardless of UV-B levels, the highest concentration of selenium was found in leaves, where the values were between 45 and 66 ng Se/g. In buckwheat leaves 44.5–63.6 mg/100 g d.m. of fagopyrin was found, and in stems 14.3–26.4 mg/100 g d.m.; here no influence of seed soaking solution or UV-B exposure was found. The content of total flavonoids in leaves was 7.8–15.9% and in stems 1.4–4.1%.

1. Introduction

Buckwheat (*Fagopyrum esculentum*) herb is used for herbal medicinal products, for green buckwheat tea, for producing buckwheat green leaf flour as an additive to some food products, and the fresh green plant parts are used as a vegetable (Hinneburg & Neubert Reinhard, 2005; Kreft, Fabjan, & Yasumoto, 2006). Buckwheat herb is especially known as a rich source of flavonoids (Kreft et al., 2006). Besides flavonoids it also contains fagopyrin, a substance similar to hypericin (of *Hypericum perforatum*), which causes light sensitivity after the ingestion of large amounts of the green parts of buckwheat (Hinneburg & Neubert Reinhard, 2005). Quinones, like hypericin and fagopyrin, express a light dependent activity; they may be used in medicine as potential sensitizers in photodynamic therapy (Ebermann, Alth, Kreitner, & Kubin, 1996). Differential extraction of flavonoids and fagopyrin from the green parts of buckwheat is possible by adjustment of the extraction conditions (Hinneburg & Neubert Reinhard, 2005). Data on the fagopyrin content in buckwheat leaves or stems are very scarce in the literature.

UV-B radiation acts as an enhancement factor for the synthesis of flavonoids in buckwheat, but too intense UV-B radiation may harm the plants and results in a lower concentration of flavonoids (Kreft, Štrukelj, Gaberščik, & Kreft, 2002). Selenium can increase the tolerance of plants to UV-induced oxidative stress (Valkama, Kivimäenpää, Hartikainen, & Wulff, 2003). Nowak, Kaklewski, and Ligocki (2004) found applied selenium has a high impact on the activity of oxidoreductase enzymes in wheat plants. The lowest selenium concentration (0.05 mmol/kg soil) positively affected antioxidant defence in wheat plants, but higher concentrations provoked stress responses. When organisms are stressed and demand more energy, ATP production and O_2 consumption are increased in the mitochondria (Bartoli, Gomez, Gergoff, Guiamet, & Puntarulo, 2005; Packard, 1985). The respiratory potential can be measured via the terminal electron transport system activity in mitochondria.

Spraying of plants with selenium solution may cause the enrichment of buckwheat grain with valuable selenium compounds in nutritionally important concentrations (Smrkolj, Stibilj, Kreft, & Germ, 2006; Stibilj, Kreft, Smrkolj, & Osvald, 2004). However, the possibilities of enhancing the concentration of selenium in buckwheat herb by soaking seeds in selenium solutions, the relation between the concentrations of selenium and phenolic substances in the plants, and interactions with UV-B radiation were not studied yet.

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The aim of this study was to investigate the impact of different levels of UV-B radiation on the respiratory potential, yield and distribution of selenium and phenolic substances in buckwheat plants obtained from seeds soaked in various concentrations of selenite and selenate solutions.

2. Materials and methods

2.1. Selenium treatment and preparation of plant samples

Common buckwheat (*F. esculentum*), cv. Darja seeds, with an average 26 g TGW (thousand grain weight), containing 22 ng Se/g seeds, were soaked for 4 h in 500 mL distilled water (MilliQ), or in solutions of sodium selenate (5, 10 or 20 mg Se^{VI}/L), or in solutions of sodium selenite (10 or 20 mg Se^{IV}/L). On average, 14.7 mg of water was absorbed by one seed after 4 h of soaking. Under the assumption that selenium was taken in by the seed with the same diffusion coefficient, the seed absorbed, with increasing concentration of selenium in the soaking solution, 74 ng, 148 ng and 295 ng of selenium per seed, respectively. After soaking, the seeds were sown in selenium-poor soil (less than 0.1 mg Se/kg soil), to which the organic fertilizer Humovit® (Cinkarna Celje; containing torf, a synthetic organic resin foam, silicious sand, and about 9 g/kg N, 15 g/kg P_2O_5 and 2 g/kg K_2O, with less than 0.1 mg Se/kg fertilizer) was added, in an outdoor research plot (Botanical Garden, University of Ljubljana: altitude 320 m above sea level, 46°35′N, 14°55′E) on 30 May 2005. Cultivation lasted until 30 August 2005. Pots (15 × 15 × 15 cm) were used for sowing. Four replicates for selenium non-treated plants for each UV-B exposure and six replicates for selenium treated plants for each UV-B exposure were made. 5–7 plants were used for each replicate.

2.2. Growth conditions

The UV-B exposure system was designed as described by Björn and Teramura (1993). In an experiment three different levels of UV-B radiation were used: (1) enhanced UV-B radiation, which simulates 17% depletion of the ozone layer, was produced by Q-Panel UV-B 313 lamps (Cleveland, OH, USA), wrapped in cellulose diacetate filters, which eliminate UV-C radiation (wavelength lower than 280 nm); (2) ambient radiation (control system) with Q-Panel UV-B 313 lamps filtered with Mylar foil, which eliminates wavelengths below approximately 320 nm; and (3) reduced ambient UV-B radiation using Mylar foil. The simulation of 17% ozone depletion and the control system consisted of six Q-Panel UV-B 313 lamps fixed in an aluminium frame (2.0 × 1.2 m) and was timer controlled. UV-B doses were calculated and adjusted weekly using the program published by Björn and Murphy (1985) and is based on the generalized plant action spectrum of Caldwell (1968). Plants were harvested when they were in the phase of seed development and maturation.

2.3. Determination of terminal electron transport system (ETS) activity

The respiratory potential of mitochondria was measured via the terminal electron transport system (ETS) activity, as described by Packard (1971). It was measured from 21 to 27 of June, 2005, at the time of flowering, on the first, fully developed leaves. Leaves of known fresh weight were crushed in a mortar in chilled 0.1 M sodium phosphate buffer (pH 8.4) containing 0.15% (w/v) polyvinyl pyrrolidone, 75 μM $MgSO_4$, and 0.2% (v/v) Triton-X-100, and homogenized by ultrasound. Then the extract was centrifuged in a refrigerated centrifuge (2K15, Sigma, Osterode, Germany) at 8500 g for 4 min at 0 °C. After that 1.5 mL substrate solution (0.1 M sodium phosphate buffer (pH 8.4), 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100), and 0.5 mL of INT (20 mg 2-*p*-iodo-phenyl 3-*p*-nitrophenyl 5-phenyl tetrazolium chloride in 10 mL of bidistilled water) were added to 0.5 mL of the supernatant. The mixture was incubated at 20 °C for 40 min. After stopping the reaction with stopping solution (formaldehyde and phosphoric acid, 1:1), the formazan absorbance at 490 nm was determined. ETS activity was calculated as the rate of INT reduction, which was converted to the amount of oxygen utilised per mg of leaf dry mass (DM) per unit time (Kenner & Ahmed, 1975).

2.4. Selenium and phenolics determination

Leaves, stems and seeds of each plant were weighed separately and then lyophilized, using a Christ Alpha 1–4, LOC-1 lyophiliser. Then the composite samples of leaves, stems and seeds of plants for each UV-B exposure were combined and before analysis milled with a Pulverisette Mill 7 and homogenized.

2.4.1. Se determination

Se content in leaves, stems and seeds samples was determined by hydride generation atomic fluorescence spectrometry (HG-AFS) (Smrkolj & Stibilj, 2004). Digestion of samples was carried out in closed teflon tubes under the following conditions: To 0.150–0.200 g of plant material, 1.5 mL HNO_3 and 0.5 mL H_2SO_4 were added. Tubes were heated for 60 min at 130 °C in an aluminium block. H_2O_2 (2 mL) was added and the samples were reheated at 115 °C for 10 min. Then 0.1 mL 40% HF was added (only to leaves and stem samples) and heated at 115 °C for 10 min. Again 2 mL of H_2O_2 was added and the tube heated at 115 °C for 10 min. After digestion, the solutions were cooled to room temperature and 0.1 mL of V_2O_5 in H_2SO_4 was added and the tubes were heated at 115 °C for 20 min. Reduction of Se^{VI} to Se^{IV} with 2.5 mL of conc. HCl was carried out at 100 °C for 10 min. Samples were diluted and selenium was determined by HG-AFS. Optimal measurement conditions are as follows: carrier flow rate 1 mL/min, argon flow rate 260 mL/min, nitrogen flow rate 3 mL/min, concentration of $NaBH_4$ 1.2% *m*/*v*, concentration of HCl for hydride generation 2 mol/L and concentration of HCl in the carrier 2 mol/L. A schematic presentation of the procedure was published by Stibilj, Mazej, and Falnoga (2003). Each sample was analysed in triplicate.

The accuracy and precision of the method was checked by analysis of the certified reference material trace element in Spinach Leaves, NIST 1570a. Good agreement was found between the obtained and certified values of 119 ± 8 ng Se/g (four determinations) and 117 ± 9 ng Se/g, respectively.

2.4.2. Determination of phenolic substances and fagopyrin

Total flavonoid and tannin contents were determined spectrophotometrically using $AlCl_3$ and vanillin–HCl reagents, respectively, as previously reported (Kreft et al., 2002). Briefly: 20 mg of powdered buckwheat sample was extracted in 10 mL of 60% ethanol overnight on a shaker. For determination of flavonoids the sample was diluted 1:6 with 60% ethanol and 20 μL of 5% $AlCl_3$ in methanol or 20 μL of methanol was added to 180 μL of diluted sample. After 30 min, the absorbance at 420 nm was measured in both solutions. The concentration was calculated from the differences of both measurements. For determination of tannins, 100 μL of 4% vanillin in ethanol or 100 μL of ethanol was added to 50 μL of undiluted sample, followed by the addition of 50 μL of concentrated hydrochloric acid. The absorbance at 500 nm was measured in both solutions and the concentration of tannins was calculated from the differences of both measurements. Each sample was analysed in duplicate. The average difference between the two measurements was 15%.

Fagopyrin content was determined by a modified method of the European European Pharmacopoeia (2002) as follows: 80 mg of powdered sample was extracted by 6 mL of 80% tetrahydrofuran in water at 65 °C for 30 min. The samples were centrifuged at 5000 rpm for 10 min (Centric 150, Tehtnica, Železniki, Slovenia, rotor: RA12), the supernatant was transferred into a fresh test tube and the sediment was extracted once more. After centrifugation, the supernatant was added to the supernatant of the first extraction. The combined extract (250 μL) was transferred to a plastic microcentrifuge tube and evaporated under vacuum (SpeedVac®). The sediment was dissolved with 500 μL of methanol in an ultrasonic bath and then centrifuged at 12000 rpm for 10 min (Centric 150, Tehtnica, Železniki, Slovenia, rotor: RA24 M). 300 μL of the supernatant was transferred to a microtitre plate vial and the absorbance was measured at 590 nm. Each sample was analysed in duplicate. The average difference between the two measurements was 17%.

2.5. Statistical analyses

The data were evaluated by multifactor ANOVA (Statgraphics Version 4) and significance accepted at $P < 0.05$.

3. Results and discussion

3.1. Terminal electron transport system (ETS) activity

Different levels of UV-B radiation did not affect ETS activity in plants from seeds soaked in distilled water (Fig. 1). Breznik, Germ, Gaberščik, and Kreft (2005) reported a lower ETS activity in common and tartary buckwheat in the time of intensive growth under enhanced UV-B radiation in selenium treated and untreated plants. Plants from seeds soaked in 20 mg Se^{IV}/L had a higher ETS activity in comparison to plants from seeds soaked in distilled water under a reduced level of UV-B radiation, while there were no significant differences between plants from seeds soaked in 20 mg Se^{IV}/L solution and plants from seeds soaked in distilled water in ambient conditions. ETS activity in plants from seeds soaked in 20 mg Se^{IV}/L solution was lowered proportionally with UV-B exposure. There are no data concerning the effect of Se^{IV} on the respiratory potential of plants. When plants are under stress, the energy demand increases (Amthor, 1995). This phenomenon was shown in the case of plants from seeds soaked in 20 mg Se^{IV}/L solution under reduced levels of UV-B radiation. When the stress is too strong for plants to overcome, the respiratory potential drops, as happened in plants from seeds soaked in 20 mg Se^{IV}/L in ambient conditions and elevated UV-B radiation (Fig. 1A). Plants from seeds soaked in distilled water were exposed to one stress – UV-B, while plants from seeds soaked in 20 mg Se^{IV}/L were exposed to both stress factors which resulted in lower ETS activity.

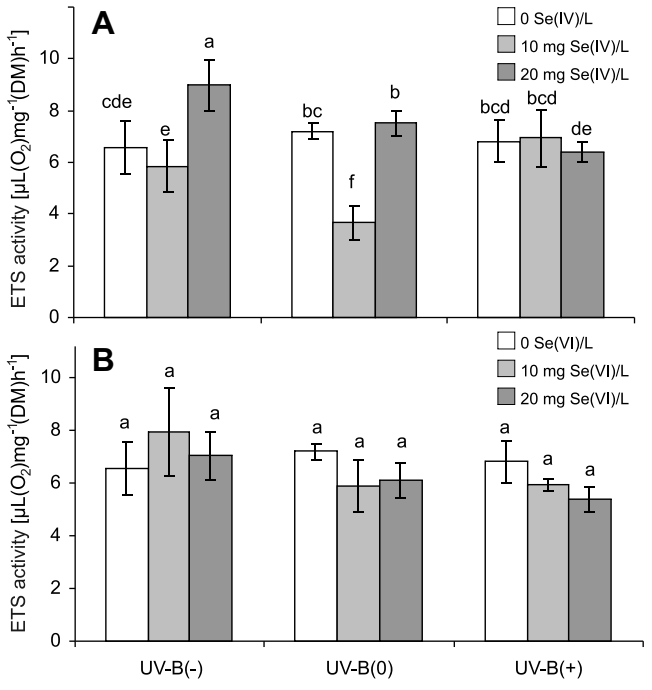

Fig. 1. Terminal electron transport system (ETS) activity in common buckwheat exposed to different levels of UV-B radiation and grown from seeds soaked in Se^{IV} solutions, expressed on a dry mass basis (Graph A). Terminal electron transport system (ETS) activity in common buckwheat exposed to different levels of UV-B radiation and grown from seeds soaked in Se^{VI} solutions, expressed on a dry mass basis (Graph B). Different letters indicate values significantly different at $P < 0.05$. Results are presented as means ± standard deviation. Error bars show 95% confidence intervals. UV-B(−) – reduced, UB-B(0) – ambient and UV-B(+) – enhanced UV-B radiation.

UV-B radiation and Se^{VI} treatment did not affect ETS activity significantly (Fig. 1B). Se^{VI} did not have a significant effect on respiratory potential since it is less toxic to plants than Se^{IV}. LeDuc et al. (2006) reported that Se^{IV} is much more toxic than Se^{VI}; seedlings of *Brassica juncea* died or were extremely stressed when exposed to selenite concentrations greater than 150 μM. Se^{VI} is much more easily metabolised in plants than Se^{IV} or organic Se (Zayed, Lytle, & Terry, 1998).

3.2. Yield parameters

Plants cultivated under reduced UV-B radiation and grown from seeds soaked in 5 mg Se^{VI}/L solution achieved the greatest height (Table 1). Notwithstanding the kind of solution used for soaking seeds, the height of buckwheat plants grown under ambient UV-B radiation was around 60 cm. Both plants from water-soaked and from selenium-enriched seeds achieved a higher height under reduced UV-B radiation, and a lower height under enhanced UV-B radiation compared to ambient UV-B conditions. The influence of selenium on plant height was observed only in the case of reduced UV-B radiation. The height of common buckwheat decreased with increasing concentration of sodium selenate (5, 10, 20 mg Se^{VI}/L) in the soaking solutions as 106, 71, and 67 cm, respectively. The same tendency was observed in buckwheat which was grown from seeds soaked in solutions containing of 10 and 20 mg Se^{IV}/L, where the values were 67 and 60 cm, respectively.

Breznik et al. (2005) studied the influence of UV-B radiation on the growth of Se-enriched common buckwheat (*F. esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). They observed a lower height in plants grown under UV-B light corresponding to 17% ozone depletion compared to ambient UV-B radiation, regardless of the plant species. Mark and Tevini (1997) observed a lower height in sunflower and maize seedlings which were grown under increased UV-B radiation of 30%. Ballaré, Scopel, Stapelton, and Yanovsky (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.

Reduced and enhanced UV-B radiation caused an increase in the mass of leaves per plant in buckwheat obtained from seeds soaked in distilled water or in solutions of sodium selenate, compared to ambient UV-B radiation (Table 1). Enhanced UV-B radiation caused an approximately factor of 2 higher mass of leaves per plant compared to ambient UV-B radiation, except in leaves of plants obtained from seeds soaked in a solution of 10 mg Se^{VI}/L. In this case only a slight increase in the leaf mass was found. A significant decrease in leaf mass under reduced UV-B radiation was found in

Table 1
The height of plants, and the mass of leaves, stems, and seeds per plant (mean ± SD), from different solutions used for soaking seeds before germination and under different levels of UV-B radiation

Solution for soaking seeds	Growth condition	Plant height (cm)	Mass of aboveground parts per plant (g)			
	UV-B radiation[a]		Leaves	Stems	Seeds	Total aboveground mass (g)
Water	B(−)	91 ± 12	0.58 ± 0.30	2.28 ± 0.80	0.58 ± 0.38	3.44 ± 0.93
	B(0)	60 ± 12	0.21 ± 0.06	2.12 ± 0.43	0.39 ± 0.10	2.72 ± 0.45
	B(+)	53 ± 8	0.39 ± 0.18	1.10 ± 0.13	0.26 ± 0.06	1.75 ± 0.23
5 mg Se^{VI}/L	B(−)	106 ± 13	1.14 ± 0.43	3.22 ± 1.20	0.63 ± 0.31	4.99 ± 1.31
	B(0)	53 ± 8	0.10 ± 0.02	1.15 ± 0.19	0.23 ± 0.04	1.48 ± 0.20
	B(+)	58 ± 11	0.20 ± 0.02	1.39 ± 0.18	0.16 ± 0.10	1.75 ± 0.21
10 mg Se^{VI}/L	B(−)	71 ± 14	0.32 ± 0.21	1.28 ± 0.48	0.31 ± 0.28	1.91 ± 0.60
	B(0)	57 ± 8	0.17 ± 0.05	1.22 ± 0.28	0.19 ± 0.04	1.58 ± 0.29
	B(+)	54 ± 10	0.19 ± 0.13	1.07 ± 0.63	0.18 ± 0.20	1.44 ± 0.67
20 mg Se^{VI}/L	B(−)	67 ± 15	0.46 ± 0.49	1.50 ± 0.22	0.41 ± 0.23	2.37 ± 0.58
	B(0)	61 ± 7	0.18 ± 0.04	1.40 ± 0.42	0.24 ± 0.05	1.82 ± 0.42
	B(+)	60 ± 14	0.31 ± 0.28	1.21 ± 0.78	0.15 ± 0.10	1.67 ± 0.83
10 mg Se^{IV}/L	B(−)	67 ± 13	0.24 ± 0.14	1.31 ± 0.23	0.30 ± 0.03	1.85 ± 0.27
	B(0)	54 ± 8	0.17 ± 0.04	1.74 ± 0.05	0.25 ± 0.11	2.16 ± 0.13
	B(+)	53 ± 12	0.14 ± 0.11	1.41 ± 0.33	0.14 ± 0.07	1.69 ± 0.35
20 mg Se^{IV}/L	B(−)	60 ± 8	0.29 ± 0.17	1.50 ± 0.38	0.36 ± 0.17	2.15 ± 0.45
	B(0)	59 ± 8	0.19 ± 0.05	1.28 ± 0.30	0.28 ± 0.04	1.75 ± 0.31
	B(+)	57 ± 7	0.12 ± 0.04	1.02 ± 0.23	0.14 ± 0.07	1.28 ± 0.24

Number of replicates was at least four.
[a] B(−) – reduced, B(0) – ambient and B(+) – enhanced UV-B radiation.

buckwheat obtained from seeds soaked in a solution containing 10 mg Se^{VI}/L compared to 5 mg Se^{VI}/L. On the other hand, enhanced UV-B radiation caused a reduction in the mass of stems per plant compared to ambient UV-B radiation, except in plants grown from seeds soaked in 5 mg Se^{VI}/L. In general, the highest mass of aboveground parts of plants was found in buckwheat which was grown under reduced UV-B radiation. In conditions of reduced UV-B radiation, the highest yield of aboveground parts of plants was obtained from the seeds treated with the lowest concentration of selenate (5 mg Se^{VI}/L), followed by plants obtained from seeds soaked in distilled water.

Treatments of seeds with Se^{IV} solutions caused a decrease in the total aboveground mass and of the respective parts, in comparison to plants obtained from seeds soaked in distilled water (Table 1). Dealing with Se^{IV} from seed soaking solutions thus represents a burden for plants.

The percentage of dry mass of leaves of buckwheat, grown under ambient UV-B radiation was independent of the soaking solution and was in the range of 22–25% (Table 2). Buckwheat which was exposed to reduced UV-B radiation showed a somewhat lower percentage of dry mass of leaves (9–22%). We also observed that in the leaves the highest proportion of dry mass was found in selenium non-treated buckwheat which was grown under enhanced UV-B radiation, namely 46% (Table 2).

On the other hand, with increasing concentration of sodium selenate in the solutions used for soaking seeds, the percentage of dry mass of leaves decreased in plants exposed to enhanced UV-B radiation as follows: 39%, 24%, and 11%. Application of sodium selenite to soak buckwheat seeds was also connected with the expression of the same tendency. The percentage of dry mass of stems (11–31%) was not dependent on soaking the seeds with selenium or UV-B conditions; the dry mass proportion of seeds was high, regardless of selenium treatment and UV-B conditions. The significant increase of transpired water in plants exposed to UV-B radiation further influenced development of common buckwheat (Gabersèik, Vončina, Trošt, Germ, & Björn, 2002). Both changes in photosynthetic and transpiration rates resulted in a strong decrease of water use efficiency (WUE). The higher transpiration rate in leaves was likely a consequence of the rigidity of the stomata or

Table 2
Dry matter of aboveground parts of plants (mean ± SD) from different solutions used for soaking seeds before germination and under different levels of UV-B radiation

Solution for soaking seeds	Growth conditions	Dry matter (%)		
	UV-B[a] radiation	Leaves	Stems	Seeds
Water	B(−)	13 ± 7	19 ± 3	52 ± 15
	B(0)	25 ± 2	19 ± 2	57 ± 18
	B(+)	46 ± 7	18 ± 4	62 ± 7
5 mg Se^{VI}/L	B(−)	20 ± 10	14 ± 8	65 ± 5
	B(0)	24 ± 13	11 ± 3	40 ± 5
	B(+)	39 ± 3	17 ± 8	60 ± 1
10 mg Se^{VI}/L	B(−)	9 ± 3	17 ± 2	67 ± 1
	B(0)	23 ± 1	31 ± 2	40 ± 19
	B(+)	24 ± 1	22 ± 5	53 ± 33
20 mg Se^{VI}/L	B(−)	19 ± 1	19 ± 3	63 ± 3
	B(0)	22 ± 2	19 ± 2	68 ± 5
	B(+)	11 ± 3	20 ± 6	88 ± 6
10 mg Se^{IV}/L	B(−)	22 ± 2	21 ± 6	64 ± 3
	B(0)	23 ± 2	18 ± 1	75 ± 14
	B(+)	42 ± 13	18 ± 1	86 ± 12
20 mg Se^{IV}/L	B(−)	18 ± 1	16 ± 7	58 ± 6
	B(0)	24 ± 4	20 ± 3	51 ± 25
	B(+)	39 ± 2	16 ± 2	71 ± 20

Number of replicates was at least four.
[a] B(−) – reduced, B(0) – ambient and B(+) – enhanced UV-B radiation.

disturbances in the permeability of membranes in guard cells (Gabersèik et al. 2002). Kyparissis, Drilias, Petropoulou, Grammatikopoulos, and Manetas (2001) showed that at the final harvest, UV-B significantly increased the stem dry mass of *Ceratonia siliqua*. Under ambient radiation selenium addition did not change the dry mass of strawberry, but in combination with UV-B radiation, decreased the dry mass of runners significantly (Valkama et al., 2003). Breznik et al. (2005) observed that UV-B radiation caused a decrease in aboveground dry mass only in selenium untreated common and tartary buckwheat. Tartary buckwheat expressed a higher aboveground dry mass under enhanced UV-B radiation in comparison to ambient UV-B radiation (Breznik, Germ, Gaberščik, & Kreft, 2004).

3.3. Selenium distribution in the aboveground parts of plants

The selenium content was determined in buckwheat leaves, stems and seeds and reported on dry matter basis (Table 3). In plants obtained from seeds soaked in water, regardless of UV-B levels, the highest concentration of selenium was found in leaves, and the values were between 49 and 66 ng Se/g. Stems and seeds accumulated less selenium. Regardless of the form of selenium, higher amounts of selenium were found in leaves and seeds than in stems. Additionally, plants obtained from seeds soaked in sodium selenate solutions accumulated more selenium in all aboveground parts than plants from seeds soaked in sodium selenite solutions. Irrespective of UV-B conditions the amount of selenium in buckwheat leaves, stems and seeds increased linearly with the increase of concentration of sodium selenate in the solution used for soaking seeds. The same relation was also observed in leaves of buckwheat plants obtained from seeds soaked in sodium selenite solution, but on a lower level of mass fraction of selenium. It was also observed that the selenium concentration in buckwheat leaves exposed to enhanced UV-B radiation was twice as much as in buckwheat leaves exposed to reduced UV-B radiation. In the case of plants from seeds soaked in 20 mg Se^{VI}/L solution, they accumulated 99 (reduced radiation) or 185 ng Se/g (enhanced radiation), and plants from seeds soaked in 20 mg Se^{IV}/L, accumulated in leaves 54 (reduced radiation) or 103 ng Se/g (enhanced radiation). Plants from seeds soaked in solution containing 20 mg Se^{VI}/L, and exposed to enhanced UV-B radiation, had the highest amount of selenium. Values for leaves, stems and seeds were 185, 135, and 363 ng Se/g, respectively. Only the seeds of buckwheat plants obtained from seeds soaked in solution containing 20 mg Se^{IV} had slightly higher amounts of selenium compared to seeds of plants from seeds soaked in distilled water. Smrkolj et al. (2006) reported higher selenium contents in flowers of tartary and common buckwheat after foliar spraying with 15 mg Se^{VI}/L in the flowering period under enhanced UV-B radiation compared to ambient UV-B radiation. The same tendency was observed in Se-enriched pumpkin (*Cucurbita pepo* L.) after addition of selenium via leaves and growth under various UV-B conditions. Pumpkins exposed to ambient radiation accumulated more selenium than plants which were grown under Mylar foil (Smrkolj, Kreft, Kapolna, & Stibilj, 2005). It seems that selenium protects plants from oxidative stress caused by UV-B radiation (Germ, Kreft, & Osvald, 2005). Pumpkins (*C. pepo* L.) grown in the field have been shown to be sensitive to ambient UV-B radiation, resulting in significantly reduced yields of fruit. Foliar addition of selenium, on the other hand, counteracted this effect, resulting in a significant increase in the yield of fruits in plants exposed to solar radiation (Germ et al., 2005).

Table 3
Mass fraction of selenium (mean ± SD) from different solutions used for soaking seeds before germination and under different levels of UV-B radiation

Solution for soaking seeds	Growth conditions	Mass fraction of selenium (ng/g dry matter)[b]		
	UV-B radiation[a]	Leaves	Stems	Seeds
Water	B(−)	49 ± 3	21 ± 3	20 ± 1
	B(0)	66 ± 1	23 ± 1	38 ± 3
	B(+)	57 ± 3	28 ± 3	31 ± 2
5 mg Se^{VI}/L	B(+)	48 ± 1	24 ± 2	25 ± 3
	B(−)	67 ± 7	30 ± 5	43 ± 2
	B(0)	73 ± 3	32 ± 5	67 ± 1
10 mg Se^{VI}/L	B(−)	75 ± 4	39 ± 5	70 ± 5
	B(0)	90 ± 5	66 ± 4	100 ± 7
	B(+)	108 ± 7	76 ± 3	190 ± 3
20 mg Se^{VI} /L	B(0)	99 ± 3	74 ± 3	122 ± 8
	B(+)	142 ± 8	99 ± 6	212 ± 6
	B(−)	185 ± 4	135 ± 4	363 ± 7
10 mg Se^{IV}/L	B(+)	30 ± 1	10 ± 1	27 ± 1
	B(−)	41 ± 2	14 ± 1	35 ± 2
	B(0)	49 ± 2	13 ± 2	40 ± 5
20 mg Se^{IV}/L	B(−)	54 ± 8	17 ± 4	37 ± 4
	B(0)	61 ± 2	18 ± 3	44 ± 2
	B(+)	103 ± 4	45 ± 7	81 ± 4

[a] B(−) – reduced, B(0) – ambient and B(+) – enhanced UV-B radiation.
[b] Samples were analyzed in triplicates; (mean ± SD).

3.4. Distribution of phenolic substances in the aboveground parts of plants

The content of total flavonoids (Table 4) in leaves (in the range 7–15%) was much higher in comparison to that in stems (1.4–4.1%). The situation regarding the content of tannins was similar (Table 4). The highest concentration of flavonoids was found in the leaves of plants from seeds treated with selenite under ambient and enhanced levels of UV-B radiation. The lowest level of flavonoids was found in plants grown from seeds soaked in 10 mg Se^{VI}/L. These plants were obviously well protected from stress and there was no need to synthesize and accumulate large amounts of protective substances. In plants obtained from seeds soaked in water, and in plants from seeds soaked in 20 mg Se^{VI}/L, the highest amount of flavonoids was present in leaves of plants grown under ambient radiation, followed by plants grown under the reduced UV-B, and the least under enhanced UV-B.

Kreft et al. (2002) reported the relation between rutin content in common buckwheat and three different UV-B levels: reduced, ambient and enhanced, simulating 17% ozone depletion. The highest amount of rutin was present in plants grown under ambient radiation, followed by plants grown under supplemented UV-B, and the least under reduced UV-B. The cited finding is in agreement with our results obtained for flavonoids in stems. According to our results, the highest amount of rutin was found in leaves of plants grown under ambient radiation as well the highest amount of flavonoids.

Table 4
Distribution of total flavonoids, tannins and fagopyrin in aboveground parts of plants

Solution for soaking seeds	Growth conditions	Content of flavonoids (g/100 g d.m.[b])		Content of tannins (g/100 g d.m.[b])		Content of fagopyrin (mg/100 g d.m.[b])	
	UV-B radiation[a]	Leaves	Stems	Leaves	Stems	Leaves	Stems
Water	B(−)	11.58	1.75	14.11	1.56	60.48	14.27
	B(0)	14.85	3.33	12.96	1.83	44.55	14.69
	B(+)	10.82	2.84	7.79	2.02	47.48	13.90
5 mg Se^{VI}/L	B(−)	13.08	4.10	16.41	2.46	45.79	14.93
	B(0)	11.63	4.06	10.62	2.58	50.31	26.45
	B(+)	13.07	3.01	14.78	3.30	44.51	15.34
10 mg Se^{VI}/L	B(−)	10.15	1.98	7.29	1.71	47.79	17.45
	B(0)	9.87	2.28	14.27	2.73	63.65	16.07
	B(+)	7.78	2.54	7.21	2.00	48.24	16.86
20 mg Se^{VI}/L	B(−)	11.82	3.50	10.16	2.35	55.17	16.86
	B(0)	15.94	3.23	21.97	2.75	51.86	14.65
	B(+)	11.25	3.39	8.19	2.64	61.06	15.21
10 mg Se^{IV}/L	B(−)	11.04	3.53	10.09	2.93	51.79	17.34
	B(0)	14.48	3.54	24.76	3.50	57.10	14.72
	B(+)	13.94	2.48	16.08	2.74	NA	16.07
20 mg Se^{IV}/L	B(−)	13.71	3.66	10.56	2.23	54.55	14.72
	B(0)	15.04	3.10	22.09	2.23	47.89	15.55
	B(+)	15.64	3.43	25.09	2.88	44.65	16.83

NA – not analysed. Samples were analysed in duplicate.
[a] B(−) – reduced, B(0) – ambient and B(+) – enhanced UV-B radiation.
[b] Dry matter.

The situation regarding the concentration of tannins is similar to the concentration of flavonoids, with the exception of plants obtained from seeds treated with selenite and exposed to ambient or enhanced UV-B radiation; the high content of tannins in leaves of these plants reflects the stressful situation under the severe influence of environmental factors.

Data on fagopyrin content in buckwheat leaves or stems in the literature are very scarce. In our study, 44.5–63.6 mg/100 g d.m. of fagopyrin was found in buckwheat leaves, and in stems the content was 14.3–26.4 mg/100 g d.m. Leaves contained about two- to threefold the amount of fagopyrin in comparison to stems. No systematic influence of soaking the seeds in different solutions or the level of UV-B radiation on fagopyrin content in leaves or stems could be observed.

In conclusion: To enhance the content of selenium in buckwheat, without loss of yield, the most efficient way is to soak buckwheat seeds prior to sowing in selenate solution, which is better than selenite. Soaking seeds with a low dose of selenate (5 mg/L) caused the yield of the resulting plants to double in comparison to plants from seeds soaked in water, if the plants are grown under a reduced level of UV-B radiation. However, because of the high leaf and total aboveground matter yield, the selenium in such plants is diluted to a level similar to the plants from seeds soaked in water. Higher, but still nutritionally safe, concentrations of selenium in buckwheat leaves (up to 142 ng/g d.m.) were obtained in plants from seeds soaked in a solution of 20 mg Se^{VI}/L, under ambient conditions, without any substantial loss in yield. The latter plants had about 16% of total flavonoids in dry matter, which was the highest level found in the experiment. Soaking buckwheat seeds before sowing in a 20 mg Se^{VI}/L solution is thus a method suitable for obtaining buckwheat herb in reasonably high yield with a high, but nutritionally safe, level of selenium and flavonoids.

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