

UV-B RADIATION INCREASES ANTHOCYANIN LEVELS IN COTYLEDONS AND INHIBITS THE GROWTH OF COMMON BUCKWHEAT SEEDLINGS

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The impact of short-term UV-B treatment on the content of individual flavonoids and photosynthetic pigments in cotyledons and the growth of common buckwheat (*Fagopyrum esculentum* Moench) seedlings was investigated. Seeds of four common buckwheat cultivars were germinated in darkness over a period of 4 days and acclimatized for 2 days under a 16/8 h light/dark photoperiod at 24/18 °C day/night, and exposure to 100–120 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of photosynthetically active radiation (PAR). Seedlings were divided into three batches, including two batches subjected to different doses of UV-B (5 $\text{W} \cdot \text{m}^{-2}$ and 10 $\text{W} \cdot \text{m}^{-2}$, one hour per day) for 5 days, and a control group exposed to PAR only. Exposure to UV-B increased anthocyanin levels in the cotyledons of all examined cultivars, it inhibited hypocotyl elongation, but did not affect the content of photosynthetic pigments. Flavone concentrations increased in cv. Red Corolla and Kora, remained constant in cv. Panda and decreased in cv. Hruszowska. Exposure to UV-B decreased rutin levels in cv. Hruszowska, but not in the remaining cultivars. Cultivars Hruszowska, Panda and Kora appeared to be less resistant to UV-B than Red Corolla. Higher resistance to UV-B radiation in Red Corolla can probably be attributed to its higher content of anthocyanins and rutin in comparison with the remaining cultivars.

Keywords: UV-B radiation – common buckwheat – seedling growth – flavonoid – chlorophyll

INTRODUCTION

The depletion of stratospheric ozone increases the intensity of UV radiation reaching the Earth from the Sun. Over years, extensive research has demonstrated that UV-B (280–315 nm) may affect plant growth and photosynthesis, and contribute to DNA damage and metabolic disturbances [23, 33]. Plants could respond to excessive irradiation by biosynthesizing phenylpropanoids whose presence in the upper epidermis creates a solar screen that absorbs UV before it can reach sensitive targets such as chloroplasts [23, 27]. However, individual phenylpropanoids in plants were found to respond differently to UV-B [8, 9, 22, 24]. For instance, in the leaves of *Hydrocotyle leucocephala*, quercetin derivatives accumulated under exposure to UV-B, thus

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reducing the concentrations of kaempferol glycosides and chlorogenic acid [20]. The flavonoid profile of broccoli sprouts was also modified under exposure to UV-B irradiation [21].

Common buckwheat (*Fagopyrum esculentum* Moench) is a fast-growing dicotyledonous plant rich in flavonoids which are partially responsible for its biological activity [14]. Buckwheat seedlings contain three major flavonoid classes: flavonols, flavones and anthocyanins [29]. The main flavonols are: rutin (quercetin-3-O-glucosyl-rhamnoside), quercetin-3-O-rhamnoside, quercetin-3-O-galactoside and the recently identified quercetin-3-O-galactosyl-rhamnoside [29]. The flavones present in the cotyledons of buckwheat seedling are vitexin, *iso*-vitexin, orientin and *iso*-orientin. Buckwheat seedlings also contain glycosides of cyanidin which occur mainly in the reddish hypocotyl and, in smaller amounts, in the cotyledons [14, 29]. Due to their high flavonoid content, buckwheat plant is often used in research examining the effects of UV radiation on these compounds [7, 11, 16, 24, 25, 28, 31].

Genotypic differences in responses to UV-B were observed in various species of plants, including in soybeans [32], maize [5], rice [6] and common buckwheat [30]. Higher levels of UV radiation can adversely affect the growth of common buckwheat and Tartary buckwheat [11, 12, 31].

The aim of this study was to evaluate the resistance of seedlings of four common buckwheat cultivars to UV-B radiation. This goal was achieved by determining whether UV-B affects: (1) seedling growth, (2) flavonol, flavone and anthocyanin concentrations, and (3) the chlorophyll and carotenoid content of cotyledons.

MATERIALS AND METHODS

Plant material and treatments

The experiment was performed on seedlings of four common buckwheat (*Fagopyrum esculentum* Moench) cultivars: Hruszowska, Luba, Kora and Red Corolla. Hruszowska is widely cultivated in Poland, whereas Kora, Panda and Red Corolla, which were bred from Hruszowska, are rarely cultivated. Four-day-old buckwheat seedlings were obtained according to a previously described procedure [14]. They were transferred to a controlled environment with a 16/8 h light/dark photoperiod, temperature of 24/18 °C, and light intensity of 100–120 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ provided by 400 W high-pressure sodium lamps (Plantaster 400W E40, Osram, Germany), and were grown in one-fifth Hoagland solution. After two days of incubation in these conditions, the seedlings were divided into three batches. One batch was used as the control, and the remaining two batches were exposed to supplementary UV-B radiation for 1 h, between 10 a.m. and 11 a.m., for 5 consecutive days. UV-B radiation (5 $\text{W} \cdot \text{m}^{-2}$ and 10 $\text{W} \cdot \text{m}^{-2}$) was achieved by placing the lamps at an appropriate height. The applied Philips TL 100 W/01 lamps emit a narrow UV-B waveband between 305 and 315 nm, with a maximal peak at 311 nm. UV-B irradiance was measured with a portable Photo-Radiometer HD2102.1 equipped with the LP 471 UVB probe (Delta OHM, Italy).

Hypocotyl elongation was measured to evaluate the effect of UV-B on seedling growth. It was expressed by the difference in hypocotyl length between the beginning and the end of the 5-day UV treatment. Mean elongation was determined based on measurements of 20–30 seedlings. At the end of the experiment, cotyledons were sampled from 10–20 seedlings per replicate and analyzed to determine their chlorophyll and carotenoid content. The concentrations of flavonoids (flavones, flavonols, anthocyanins) and total phenolics were determined in the remaining parts of the evaluated plants. Before analyses, plant samples were freeze-dried in a laboratory freeze dryer (Alpha 1–2 LD plus; Christ, Germany) and ground in a Tube Mill (IKA, Germany). Flavonoid and total phenolic content was expressed on a dry weight basis obtained by freeze-drying.

Determination of flavonoid concentrations

Flavonoids were analyzed according to a previously described procedure [14]. Briefly, freeze-dried and ground samples of buckwheat cotyledons were fivefold extracted by sonication with a mixture of 60% methanol and 0.4% trifluoroacetic acid. The HPLC analysis was carried out in an apparatus equipped with a Cadenza CD-C₁₈ 3 µm column, 250×2.0 mm ID, and a UV detector set at 350 nm (flavones and flavonols) and 520 nm (anthocyanins). The gradient elution system was comprised of solvent A (water/acetonitrile/formic acid, 89:6:5) and solvent B (water/acetonitrile/formic acid, 15:80:5). Most flavonoids were identified by comparing their retention times against the available standards. Flavonoid concentrations were calculated with commercial standards, excluding quercetin-3-O-galactosyl-rhamnoside and cyanidin 3-O-galactosyl-rhamnoside whose content was calculated based on the standards for rutin and cyanidin 3-rhamnosyl-glucoside, respectively. The cyanidin 3-rhamnosyl-glucoside standard was supplied by Polyphenols (Norway), whereas the standards for orientin (luteolin-6C-glucoside), *iso*-orientin (luteolin-8C-glucoside), vitexin (apigenin-8C-glucoside), *iso*-vitexin (apigenin-6C-glucoside) and rutin (quercetin-3-O-glucosyl-rhamnoside) were supplied by Extrasynthese (France). Quercetin-3-O-galactosyl-rhamnoside and cyanidin 3-O-galactosyl-rhamnoside were identified based on previously published data [29].

Other analyses

Total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent (Sigma). The samples (0.1 mL of plant extract in 60% ethanol-water) were combined with 2.4 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. The mixture was stirred, left stand for 10 minutes, and combined with 2 mL of 10% Na₂CO₃. The reaction mixture was left to stand for 2 hours in darkness, centrifuged, and its absorbance of was measured at 725 nm. Chlorogenic acid (Sigma) was used to prepare the standard curve. The content of chlorophylls and

total carotenoids was quantified spectrophotometrically based on the method and extinction coefficients proposed by Lichtenthaler and Wellburn [18]. The results were analyzed statistically by the Newman–Keuls test at $p \leq 0.05$. Mean values marked with various letters are significantly different.

RESULTS AND DISCUSSION

UV-B radiation may influence the content of secondary metabolites and plant growth [23, 33]. Those effects are determined by the applied dose, radiation quality and time of exposure. The results of our study revealed that the applied UV-B doses were detrimental to the growth of common buckwheat seedlings. Even short-term (one hour

Table 1

The effect of UV-B radiation on hypocotyl elongation and the content of chlorophylls, carotenoids and total phenolics in the cotyledons of common buckwheat cultivars. Elongation is expressed by the mean values of 10–15 replicates

	Hruszowska	Panda	Kora	Red Corolla
Hypocotyl elongation (mm)				
Control	31.1±14.0a	40.9±15.0a	35.4±12.2a	33.9±5.8a
UV-B, 5 W·m ⁻²	16.6±8.4b	27.1±8.9b	24.5±9.1b	27.9±6.2b
UV-B, 10 W·m ⁻²	20.8±9.1ab	32.8±7.6b	21.0±9.3b	24.0±6.7b
Chlorophyll <i>a</i> (mg·g ⁻¹ fresh weight)				
Control	1.30±0.13a	1.34±0.13a	1.26±0.11a	1.52±0.19a
UV-B, 5 W·m ⁻²	1.31±0.11a	1.35±0.09a	1.22±0.27a	1.50±0.21a
UV-B, 10 W·m ⁻²	1.35±0.15a	1.41±0.08a	1.05±0.21a	1.45±0.20a
Chlorophyll <i>b</i> (mg·g ⁻¹ fresh weight)				
Control	0.45±0.05a	0.50±0.07a	0.41±0.08a	0.54±0.07a
UV-B, 5 W·m ⁻²	0.47±0.08a	0.48±0.05a	0.42±0.08a	0.51±0.07a
UV-B, 10 W·m ⁻²	0.45±0.05a	0.50±0.04a	0.35±0.06a	0.52±0.07a
Total carotenoids (mg·g ⁻¹ fresh weight)				
Control	0.28±0.03a	0.28±0.02a	0.27±0.03a	0.33±0.04a
UV-B, 5 W·m ⁻²	0.28±0.02a	0.29±0.01a	0.25±0.05a	0.30±0.04a
UV-B, 10 W·m ⁻²	0.29±0.04a	0.30±0.02a	0.23±0.04a	0.31±0.04a
Total phenolics (mg·g ⁻¹ dry weight)				
Control	56.8±2.0a	52.3±3.2a	57.5±2.0a	57.5±0.9a
UV-B, 5 W·m ⁻²	60.3±2.7a	52.0±3.3a	59.3±2.9a	65.3±2.2a
UV-B, 10 W·m ⁻²	61.6±3.4a	49.7±1.7a	60.8±3.8a	59.7±3.2a

Chlorophyll concentrations and total carotenoid content are expressed by the mean values of 8 replicates, and total phenolic content – by the mean values of 3 replicates. Different letters indicate significant differences ($p \leq 0.05$) according to the Newman–Keuls test.

daily) exposure to UV-B for five consecutive days inhibited hypocotyl elongation in the seedlings of all examined cultivars (Table 1). Plant growth was most inhibited in cv. Hruszowska and Panda, and it was generally independent of the applied dose of UV-B. These results are similar to previously published data for common buckwheat and Tartary buckwheat grown in field conditions [11, 12, 31].

In the present study, UV-B radiation did not significantly influence chlorophyll and carotenoid levels in the cotyledons of buckwheat seedlings. Unfortunately, these results are presented on a fresh weight basis, which could lead to inaccurate conclusions. The content of phenolics was not significantly modified under exposure to supplementary UV radiation either. Our results are consistent with the previously published findings [4, 19]. Minor changes in the concentrations of photosynthetic pigments can probably be attributed to the short time of UV-B exposure. Chlorophyll levels were substantially reduced in buckwheat plants grown outdoors between July and October under exposure to UV-B [11]. However, a comparison between seedlings and fully mature plants is not justified.

Flavonoids protect the photosynthetic apparatus and subcellular organelles against damage caused by UV radiation [23, 25]. Their presence in the walls of epidermal cells is generally associated with UV protection, whereas the presence of mesophyll cells in vacuoles and chloroplasts is linked with reactive oxygen species scavenging [1]. According to many reports, UV-B radiation increases flavonoid levels in young plants [2, 19, 21, 25]. In this study, the concentrations of individual flavones indicate that the effect of UV-B is genetically determined (Table 2). The content of C-glycosides increased in the cotyledons of two buckwheat cultivars (Red Corolla, Kora), it remained stable in cv. Hruszowska and decreased in cv. Panda.

Buckwheat plants also contain flavonols quercetin-3-O-galactosyl-rhamnoside and quercetin-3-O-glucosyl-rhamnoside (rutin). In this experiment, the highest flavonol concentrations were noted in the cotyledons of Red Corolla. Exposure to UV-B radiation did not affect rutin levels in the cotyledons of three cultivars, and a decrease in rutin content was observed only in cv. Hruszowska in response to a higher dose of UV-B (Table 2). Under exposure to UV-B, the content of quercetin-3-O-galactosyl-rhamnoside clearly increased in cv. Red Corolla. Our results are generally consistent with the previously published data for other plants where individual phenylpropanoids responded differently to the UV-B [8, 9, 22]. For instance, UV-B stimulated the accumulation of quercetin-3,4-O-diglucoside, but not quercetin-4-O-glucoside in asparagus [8]. In *Brassica oleracea*, UV-B specifically induced the accumulation of kaempferol-3-O-disinapoyl-triglucoside-7-O-glucoside, but decreased the levels of other derivatives of kaempferol and quercetin [22]. In *Primula veris* leaves, UV had no effect on total flavonoid levels, but it exerted a varied effect on flavonoids [9]. Our results also partially confirm the findings of Regvar et al. [24]. In the cited study, supplementary UV-B did not affect the rutin levels in common buckwheat plants, but it should be noted that the experiment was conducted outdoors over a long period of time. On the other hand, exposure to intense UV-B radiation may cause damage to common buckwheat plants and reduce their rutin content [16].

Table 2
The effect of UV-B radiation on the content ($\text{mg} \cdot \text{g}^{-1}$ dry weight) of flavones and flavonols in the cotyledons of common buckwheat cultivars

	Hruszowska	Panda	Kora	Red Corolla
Orientin (luteolin-8C-glucoside)				
Control	3.29±0.05a	3.02±0.06a	2.97±0.09b	2.89±0.04b
UV-B, 5 $\text{W} \cdot \text{m}^{-2}$	3.04±0.02b	na	na	2.87±0.01b
UV-B, 10 $\text{W} \cdot \text{m}^{-2}$	3.16±0.10ab	2.97±0.12a	3.57±0.14a	3.21±0.03a
<i>iso</i> -Orientin (luteolin-6C-glucoside)				
Control	5.65±0.13a	5.25±0.07a	5.25±0.09b	5.18±0.06b
UV-B, 5 $\text{W} \cdot \text{m}^{-2}$	5.25±0.03b	na	na	5.18±0.02b
UV-B, 10 $\text{W} \cdot \text{m}^{-2}$	5.52±0.11ab	5.15±0.05a	6.18±0.09a	5.69±0.10a
Vitexin (apigenin-8C-glucoside)				
Control	4.01±0.07a	3.03±0.06a	3.11±0.08b	3.23±0.06b
UV-B, 5 $\text{W} \cdot \text{m}^{-2}$	3.64±0.02b	na	na	3.32±0.02b
UV-B, 10 $\text{W} \cdot \text{m}^{-2}$	3.64±0.08b	3.21±0.05a	3.74±0.06a	3.53±0.05a
<i>iso</i> -Vitexin (apigenin-6C-glucoside)				
Control	8.02±0.15a	6.37±0.10a	6.04±0.08b	7.05±0.07c
UV-B, 5 $\text{W} \cdot \text{m}^{-2}$	7.41±0.01b	na	na	7.34±0.04b
UV-B, 10 $\text{W} \cdot \text{m}^{-2}$	7.49±0.06b	6.44±0.13a	7.56±0.12a	7.77±0.10a
Quercetin-3-O-galactosyl-rhamnoside				
Control	1.85±0.07a	1.06±0.03a	1.74±0.08a	2.05±0.09b
UV-B, 5 $\text{W} \cdot \text{m}^{-2}$	1.26±0.06b	na	na	2.02±0.02b
UV-B, 10 $\text{W} \cdot \text{m}^{-2}$	1.79±0.03a	0.96±0.03a	0.91±0.04b	2.79±0.04a
Quercetin-3-O-glucosyl-rhamnoside (rutin)				
Control	12.65±0.25a	15.16±0.08a	11.59±0.16a	25.49±0.30a
UV-B, 5 $\text{W} \cdot \text{m}^{-2}$	12.20±0.08a	na	na	25.48±0.14a
UV-B, 10 $\text{W} \cdot \text{m}^{-2}$	11.27±0.16b	14.55±0.30a	11.11±0.17a	25.99±0.06a

The results are expressed by the mean values of 3 replicates±SD. Different letters indicate significant differences ($p \leq 0.05$) according to the Newman-Keuls test; na – not analyzed.

Buckwheat cotyledons accumulate different amounts of individual flavonoids, which could explain intraspecific differences in response to UV-B. Such relationship was found in soybean cultivars where UV-B induced a significant increase in the flavonoid content of seven cultivars, a decrease in flavonoid levels in five cultivars and no changes in the remaining eight cultivars [32], in *Arabidopsis* [10] and common buckwheat [30].

Flavonoids are quickly depleted as antioxidants, and they effectively quench H_2O_2 as substrates for vacuolar peroxidases [26]. UV-B radiation has also been found to

induce the expression of phenol-oxidizing peroxidases in many plant species [15], which suggests that flavonoids are relatively rapidly oxidized. The above observation could also be attributed to the different roles of flavonoids in the epidermis and mesophylic cells of plants. There is evidence suggesting that UV-B irradiation increases the content of flavonoids in the epidermis, but not in the mesophyll [3].

In the evaluated cultivars, anthocyanin levels were highest in the cotyledons of Red Corolla, and they were approximately threefold lower in the remaining cultivars (Table 3). Red Corolla is characterized by red colored leaves and sepals. UV-B increases the accumulation of anthocyanins by stimulating the expression of genes encoding enzymes in the anthocyanin biosynthetic pathway [17]. According to the leading hypothesis, anthocyanins protect the photosynthetic apparatus from the effects of excessive incident visible radiation or UV-B radiation [13]. These general principles were confirmed in our study which demonstrated that the concentrations of cyanidin-3-O-galactosyl-rhamnoside and cyanidin-3-O-glucosyl-rhamnoside in the cotyledons of all tested buckwheat cultivars increased significantly under exposure to UV-B radiation. Similar results were previously noted in common buckwheat [7, 25, 28], Tartary buckwheat [31] and other plants [2, 19, 21].

The relationships between total anthocyanins and rutin concentrations and the differences in hypocotyl elongation between control and UV-treated seedlings were analyzed statistically. The Pearson correlation coefficients for anthocyanins (-0.828) and rutin (-0.815) were significant at $p \leq 0.05$ in a two-tailed probability test. The correlation coefficients for quercetin-3-O-galactosyl-rhamnoside, and apigenin and luteolin glucosides, calculated in a similar manner, were not statistically significant.

Table 3

The effect of UV-B radiation on the anthocyanin content ($\text{mg} \cdot \text{g}^{-1}$ dry weight) in the cotyledons of common buckwheat cultivars

	Hruszowska	Panda	Kora	Red Corolla
Cyanidin-3-O-galactosyl-rhamnoside				
Control	0.92±0.02b	0.66±0.01b	0.65±0.02b	2.54±0.03c
UV-B, 5 W·m ⁻²	0.93±0.01b	na	na	2.85±0.02b
UV-B, 10 W·m ⁻²	1.30±0.03a	0.81±0.01a	0.89±0.04a	3.17±0.07a
Cyanidin-3-O-glucosyl-rhamnoside				
Control	0.08±0.02b	0.01±0.01b	0.14±0.01b	0.74±0.02b
UV-B, 5 W·m ⁻²	0.19±0.01a	na	na	1.02±0.05a
UV-B, 10 W·m ⁻²	0.13±0.01b	0.05±0.01a	0.23±0.01a	0.93±0.02a
Total anthocyanins				
Control	1.00±0.02 c	0.67±0.01b	0.79±0.03b	3.27±0.14c
UV-B, 5 W·m ⁻²	1.12±0.01b	na	na	3.87±0.06b
UV-B, 10 W·m ⁻²	1.43±0.03a	0.86±0.01a	1.11±0.04a	4.10±0.09a

The results are expressed by the mean values of 3 replicates ±SD. Different letters indicate significant differences ($p \leq 0.05$) according to the Newman-Keuls test; na – not analyzed.

CONCLUSIONS

The present study demonstrated that short-term exposure to UV-B radiation induced intraspecific defense responses in seedlings of common buckwheat seedlings, which were manifested by various changes in the concentrations of flavones and flavonols in cotyledons. In contrast, UV-B exposure increased the anthocyanin content of all examined cultivars and inhibited elongation of seedling hypocotyls. Cultivars Hruszowska, Panda and Kora appeared to be less resistant to UV-B than Red Corolla. Higher resistance of Red Corolla to UV radiation can probably be attributed to three-fold higher concentrations of anthocyanins and twofold higher levels of rutin in comparison with the remaining cultivars.

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