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Long Wave UV-B Radiation and Asahi SL Modify Flavonoid Content and Radical Scavenging Activity of *Zea mays* var. *saccharata* Leaves

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The experiment on *Zea mays* L. cv. Landmark (F1) plants was performed in a greenhouse with UV-B (305–315 nm). The pots with plants were divided into four groups: the first and the second groups were grown, respectively, at low (1.0 kJ m^{-2} d^{-1}) and high (3.0 kJ m^{-2} d^{-1}) biological effective dose of UV-B radiation. Half of the pots of each group were sprayed with 0.1% solution of Asahi SL (the third and fourth groups). The intensity of photosynthesis and transpiration, chlorophyll fluorescence, the content of UV-absorbing compounds and radical scavenging activity were measured using DPPH after four and six weeks of UV-B radiation. After six weeks of irradiation with a higher UV-B dose both flavonoid content and antioxidant activity increased by 112% and by 44%, respectively, compared to the plants grown at the lower dose. The plants treated with Asahi SL and exposed to the high dose of UV-B had the content of flavonoids 80% higher than the control ones. Asahi SL decreased scavenging activity in both groups of plants by 17% and 32%, respectively, in comparison with the untreated plants. The intensity of net photosynthesis, the transpiration rate and chlorophyll fluorescence parameters (Fv/Fo, ETR, Rfd) did not differ in most of variants.

Keywords: biostimulator, DPPH, flavonoids, maize, photosynthesis, ultraviolet B

INTRODUCTION

UV-B radiation (280 nm – 315 nm) has long been known to damage plants by modifying metabolic processes, growth and development (Hollosy, 2002; Żuk-Gołaszewska et al., 2003; Björn, 2007; Dotto and Casati, 2017). UV-B measurements in Central Europe showed increase of 5% per decade (UNEP, 2018). Current global terrestrial UV-B radiation levels are somewhere between 0 and 12 kJ/m^2 per day on the Earth's surface (Bandurska et al., 2013; Kataria et al., 2014). One way of defending plants against UV-B radiation involves increasing UV-B absorbing pigments such as flavonoids after the actual UV-B entry into the outer layer of the leaf surface (Kakani et al., 2003; Mahdavian et al., 2008; Yin and Ulm, 2017; Mosadegh et al., 2018). Moreover, plants also have a free radicals scavenging system using enzymes such as superoxide dismutase, catalase or peroxidase. Flavonoids are also involved in neutralizing radicals (Jansen et al., 2008; Reboredo and Lidon, 2012). Some compounds referred to as biostimulators (biostimulants) may also cause better growth and development, as well as improve the physiological state of plants subjected to environmental stress factors, including UV-B radiation (Skórska, 2008; Przybysz et al., 2010). One of them is Asahi SL recommended for use on many agricultural crops to improve yield quality, and to reduce the adverse effects of stress-inducing factors (Przybysz et al., 2016). In our earlier paper we showed that Asahi SL caused increase of flavonoid content in basil plants subjected to broadband UV-B more than untreated plants (Skórska and Witczak, 2009). We expected that this biostimulant as well as UV-B could also change antioxidant activity of plants. The aim of the

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experiment was to investigate if long wave UV-B radiation modifies photosynthesis, transpiration rate, photochemical efficiency of photosystem II using the chlorophyll fluorescence method, and particularly flavonoid content and radical scavenging activity of *Zea mays* L. plants treated with Asahi SL.

MATERIAL AND METHODS

Seeds of *Zea mays* L. var. *saccharata* cv. Landmark (F1) received from the CNOS-VILMORIN Sp. z o.o in Poznań were sown in 20 pots with volume of 2 dm^3 with ca. 1.1 kg of soil (substrate of ANIA peat mixture, pH 5.5–6.5) and sand (1:1). The experiment was carried out in a greenhouse of the West Pomeranian University of Technology in Szczecin from May to July (PPFD at noon ca. 500 µmol m^{-2} s^{-1}, temperature 25°C/20°C, day/night). The source of UV-B radiation were two TL100/01 lamps (Philips, Eidhoven, the Netherlands) emitting in the range from 305 nm to 315 nm (Skórska, 2000) for 6 hours a day from 9 a.m. to 3 p.m. The intensity of UV-B radiation was measured using an IL 1403 radiometer with a SEL 240-UVB1 calibrated detector (International Light Inc., USA). Plant pots were divided into four groups: plants of the first and the second group grewat a low ($UV\text{-}B_{BE}$ = 1.0 kJ m^{-2} d^{-1}) and high (3.0 kJ m^{-2} d^{-1}) daily biologically effective UV-B dose, respectively, calculated according to Caldwell (1977). When the plants were in the phase of the fourth leaf, BBCH 18 (Meier, 2001) half of the pots of each group were sprayed with 0.1% solution of Asahi SL, composed of sodium ortho-nitrophenolate (0.2%), sodium para-nitrophenolate (0.3%) and sodium 5-nitroguaiacolate (0.1%) (Arysta Life Science Poland Ltd.). After four and six weeks of UV-B radiation the measurements were taken on fully developed 6th leaves in 5–6 biological replications (independent leaves). The net photosynthesis intensity (p_N, µmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were measured using an open circuit portable TPS-2 gas-exchange system with a portable PLC camera (PP Systems, UK). Chlorophyll fluorescence parameters (F_v/F_o, ETR, Rfd) of maize leaves were measured with a PAM 210 fluorometer (Walz, Effeltrich, Germany), as described earlier (Skórska and Murkowski, 2018). To determine UV-absorbing compounds (mainly flavonoids) the ethanol extract from leaves was prepared according to Caldwell et al. (1994). Absorption spectra in the range from 280 to 380 nm were performed using a Specord M42 spectrophotometer (Zeiss, Germany) and the flavonoid content was expressed as an absorbance at 305 nm per 1 dm^2 of leaf area (A_{305} dm^{-2}). The DPPH (2,2-diphenyl-1-picrylhydrazyl 95%, free radical, Sigma-Aldrich Co.) radical scavenging activity of the 7th leaves cut off plants in BBCH 30 phase was determined according to Yen and Chen (1995) and modified by Grzeszczuk et al. (2010). A homogenized leaf sample was mixed with methanol (80%) in a round-bottomed volumetric flask. The flask was placed in an ultrasonic cleaner for 15 minutes. The obtained mixture was transferred to a tube and centrifuged at 10°C at 10 000 rpm for 5 minutes. 1 cm^3 of the diluted sample was mixed with 3 cm^3 of methanol and 1 cm^3 of DPPH solution (0.012 g DPPH was dissolved in 100 cm^3 pure methanol; 0.3 mM). The solution (A_T) was shaken and left at room temperature in the dark for 10 min; the absorbance was measured spectrophotometrically at 517 nm. The reagent blank (A_R) contained methanol instead of the sample solution. Percent (%) inhibition of DPPH was calculated according to Rossi et al. (2003) and Ardestani and Yazdanparast (2007): % DPPH = 100 – [(A_T/A_R) × 100], where: A_T – absorbance of tested sample, A_R – absorbance of reference solution. The results were statistically analyzed by two-way ANOVA using Statistica software. To verify the significance of differences between the treatment means, a confidence interval of Newman-Keuls test was used at significance level $p < 0.05$. The results marked with the same letter do not differ significantly from each other.

RESULTS

Enhanced UV-B caused increase of ultraviolet absorbing compounds (mainly flavonoids) in the leaves by 20% after 4 weeks and by 112% after 6 weeks of irradiation in comparison with the plants grown at the lower dose (Table 1). The plants treated with Asahi SL and subjected to the lower dose of UV-B had more of these compounds after 4 and 6 weeks of irradiation, by 17% and 26%, respectively, compared to the control plants (without Asahi). The plants treated with a biostimulator and exposed to the higher dose of UV-B had more flavonoids by 62% and 52%, respectively, than the plants exposed to the lower dose of UV-B.

At the higher dose of UV-B after 4 weeks of irradiation the radical scavenging activity increased by 28%, compared with the plants grown at the lower dose of ultraviolet radiation (Fig. 1a), and by 44% after 6 weeks (Fig. 1b). Asahi SL caused a decrease in radical scavenging activity in both groups of plants by 44% and 28%, respectively, in comparison with the plants not treated with this biostimulator. After 6 weeks of exposure to UV-B the biostimulator effect was clearly weakened (Fig. 1b). The net photosynthesis intensity (p_N),

TABLE 1. The content of UV-absorbing compounds (flavonoids) of the plants untreated and treated with Asahi SL and exposed to low (1.0 kJ m^{-2} d^{-1}) and high (3.0 kJ m^{-2} d^{-1}) dose of UV-B radiation (mean ± SD). Means in the row marked with the same letters are not significantly different at $p \leq 0.05$ according to Newman-Keuls test, n = 5.

A_{305} dm^{-2}	Control (without Asahi)		With Asahi SL	
	Low UV-B	High UV-B	Low UV-B	High UV-B
After 4 weeks	19.9 c ± 1.7	23.9 b ± 2.2	23.4 b ± 2.2	37.9 a ± 3.9
After 6 weeks	15.1 b ± 1.3	32.0 a ± 4.1	19.1 b ± 3.0	29.0 a ± 5.2

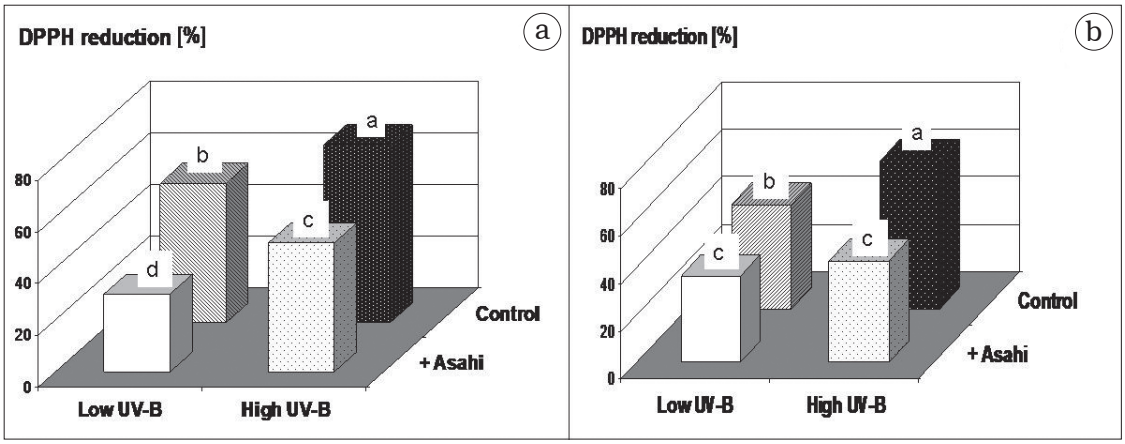

Fig. 1. Radical scavenging activity (% DPPH reduction) of the maize plants subjected to the low (1.0 kJ m^{-2} d^{-1}) and the high (3.0 kJ m^{-2} d^{-1}) dose of UV-B radiation for 4 weeks (**a**) and 6 weeks (**b**). Means on the figure marked with the same letters are not significantly different at $p \leq 0.05$ according to Newman-Keuls test, n = 3.

the transpiration rate (E) and the chlorophyll fluorescence parameters F_v/F_o (maximal quantum yield of water photolysis system of the donor side of PSII), ETR (electron transport rate) and Rfd (vitality index) in all variants did not differ significantly (Table 2).

DISCUSSION

The results obtained in our experiment showed that UV-B radiation in the applied higher biologically effective dose generated synthesis of antioxidant compounds in the maize leaves, thanks to which there were more of them than at the lower dose. Plants are able to prevent the harmful effects of UV-B radiation by synthesizing flavonoids, a class of UV-absorbing compounds located mainly in the epidermis. They act as internal filters protecting against the penetration of harmful radiation inside the cell and as antioxidants, reducing the reactive oxygen species formed under the influence of UV-B (Ardestani and Yazdanparast, 2007; Agati et al., 2012; Bandurska et al., 2013). Jansen et al. (2008) consider them a beneficial side effect of UV-B radiation, because antioxidants are desirable in the human diet and animal feed. Plants of various species growing in the presence of this radiation in the natural environment in field conditions are usually enriched with these compounds (Mahdavian et al., 2008; Agati et al., 2012; Tossi et al., 2012). Induction of the metabolic pathway of these compounds in plants is one of widely described and widespread defensive reactions to this radiation range (Holosy, 2002; Kakani et al., 2003; Redoredo and Lidon, 2012). In their experiment Carletti et al. (2003) after 3 days of exposure of maize seedlings at an early stage of development to UV-B, using the same type of lamps as in our experiment, but with a dose almost 3 times higher (8.35 kJ m^{-2} d^{-1}), observed significantly increased content of these protective compounds.

In our experiment, plants treated with Asahi SL biostimulant after 4 weeks of the higher UV-B

TABLE 2. The intensity of net photosynthesis (pN), transpiration rate (E) and chlorophyll fluorescence parameters of the plants untreated and treated with Asahi SL and exposed to low (1.0 kJ m^{-2} d^{-1}) or high (3.0 kJ m^{-2} d^{-1}) dose of UV-B radiation for 4 and 6 weeks (mean ± SD). Means in the row marked with the same letters are not significantly different at $p \leq 0.05$ according to Newman-Keuls test, n = 5.

Feature	Control (without of Asahi)		With Asahi	
	Low UV-B	High UV-B	Low UV-B	High UV-B
		After 4 weeks		
p_N [µmol·m^{-2}·s^{-1}]	15.8 a ± 1.1	14.1a ± 2.8	12.4 a ± 2.6	16.1 a ± 3.0
E [mmol·m^{-2}·s^{-1}]	1.9 a ± 0.5	1.9 a ± 0.4	1.8 a ± 0.7	1.6 a ± 0.6
F_v/F_o	3.05 a ± 0.04	3.05 a ± 0.19	3.23 a ± 0.15	3.03 a ± 0.19
ETR	29.0a ± 0.6	29.3 a ± 1.2	28.7 a ± 1.1	28.4 a ± 1.7
Rfd	1.88 a ± 0.06	1.98 a ± 0.11	1.91 a ± 0.06	1.92 a ± 0.09
		After 6 weeks		
p_N [µmol·m^{-2}·s^{-1}]	18.8 a ± 3.2	17.6 a ± 2.1	20.3 a ± 1.4	21.1 a ± 1.4
E [mmol·m^{-2}·s^{-1}]	2.9 a ± 0.3	2.8 a ± 0.1	2.9 a ± 0.3	3.2 a ± 0.1
F_v/F_o	4.57 a ± 0.41	4.33 a ± 0.34	4.69 ± 0.35	4.68 ± 0.13
ETR	30.9 a ± 0.8	30.9 a ± 1.3	31.1 a ± 0.5	31.7 a ± 0.8
Rfd	2.88 a ± 0.12	2.81 a ± 0.36	2.73 a ± 0.19	2.99 a ± 0.22

dose contained from 17% to 62% more protective compounds in comparison with the control. The radical scavenging activity of the leaves treated with Asahi SL was lower than in the untreated plants. In the experiment described by Wysocki et al. (2017) the radical scavenging activity of strawberry cv. Kent fruits treated with Asahi slightly decreased in comparison with the control ones. It is possible that ultraviolet radiation in connection with compounds included in Asahi caused decomposition of antioxidant compounds created under the influence of ultraviolet radiation.

Our results regarding photosynthesis, leaf transpiration intensity and chlorophyll fluorescence parameters reflecting functioning of Photosystem II did not significantly change under the influence of the higher UV-B dose, which may confirm the tolerance of this species to the applied UV-B dose. The photosynthetic apparatus is one of main goals of this radiation, and its damage leads in the final effect, namely a reduction in yield (Szilárd et al., 2007; Kataria et al., 2014). Some researchers regard maize to be a tolerant species, adapted to high ultraviolet irradiance (Carletti et al., 2003; Lau et al., 2006), however others observed a decrease in the net photosynthesis rate. Shen et al. (2015) showed that maize seedlings exposed to 5.4 kJ m^{-2} d^{-1} of biologically effective UV-B radiation for 10 days had a decreased net photosynthesis rate from ca. 12 to 4.5 µmol CO_2 m^{-2} s^{-1}, while the transpiration rate was unchanged.

Plant reactions depend on the development phase - at the early stages they are usually more susceptible than the developed ones (Bjorn, 2007; Prado et al., 2012). They also depend on the applied dose and a source of radiation, as well as on a ratio of UV-B intensity to photosynthetically active radiation (PAR). In the described experiments, the plants often grew at a high level of UV and a low level of PAR, unlike in natural or greenhouse conditions. Reboredo and Lidon (2012) concluded that photosynthesis is not significantly affected by UV-B radiation when plants grow in natural conditions. Similar results were presented by Lau et al. (2006) regarding a maize line with genetically increased content of flavonoids - the net photosynthesis rate did not change at UV-B dose of ca. 9 kJ m^{-2} d^{-1}. Measured by us chlorophyll fluorescence parameters Fv/Fo, ETR and Rfd did not change either under the influence of a higher dose of UV-B or with the use of a biostimulator. These results also confirm relatively high tolerance of maize plants to the applied dose of UV-B radiation.

In another experiment carried out by us on cucumber plants at the UV-B dose of 3.0 kJ m^{-2} d^{-1} applied for 7 days, F_v/F_o decreased by 41% compared to control plants, indicating damage to

the oxygen evolving complex (OEC), while in the plants treated with a NanoGro® biostimulator the F_v/F_o value did not change (Skórska, 2008). In the plants exposed to UV-B and treated with this biostimulator, no changes of the flavonoids content were found.

The results of some research show positive effects of Asahi SL. Przybysz et al. (2016) described the beneficial effect of this biostimulator in the case of *Arabidopsis thaliana* L. plants treated with cadmium, concerning the rate of CO_2 assimilation and the transpiration.

CONCLUSIONS

1. A clear effect of the higher UV-B dose on the ultraviolet compounds content (flavonoids) by 20% and by 112% was observed, compared to the plants grown at lower UV-B after four and six weeks of the irradiation, respectively; in the plants treated with Asahi SL the content of these compounds was higher by 62% and 52%.
2. At the higher dose of UV-B after 4 weeks of irradiation the radical scavenging activity increased by 28%, compared to the plants grown at the lower dose and by 44% after 6 weeks. Asahi SL caused decrease in the antioxidant activity in both groups of plants by 17% and 32%, respectively, in comparison with the untreated plants.
3. There were no significant changes in photosynthesis intensity, transpiration rate and chlorophyll fluorescence indicating Photosystem II functions in all variants.

AUTHORS' CONTRIBUTIONS

The authors of this article have contributed equally, and declare that there are no conflicts of interest.

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