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Response of Several Spring Barley Cultivars to UV-B Radiation and Ozone Treatment

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Experiments were conducted under controlled environmental conditions to assess the impact of UV-B radiation (8 kJ m^{-2} d^{-1}) and ozone (240 µg m^{-3}) on seven spring barley (*Hordeum vulgare* L.) cultivars. It has been established that the impact of UV-B and ozone on various spring barley cultivars differs greatly and in most cases the cultivars which are tolerant to an impact of ozone are sensitive to UV-B and vice versa. The impact of both investigated stressors resulted in a more pronounced decrease in chlorophyll *a* than chlorophyll *b* content while the chlorophyll *a/b* ratio in the leaves of different spring barley cultivars has also decreased. The reaction of carotenoids differs greatly depending on a stressor. UV-B radiation causes a significant increase in the content of carotenoids which is most likely a defence reaction against high energy short-wave radiation. The impact of ozone, in contrast, resulted in a decrease in carotenoids in the leaves of different spring barley cultivars.

Key words: *chlorophylls, carotenoids, dry biomass, spring barley, ozone, UV-B radiation.*

1. Introduction

Intensity of solar UV-B radiation to the Earth surface, especially at higher latitudes, has increased as a result of stratospheric ozone depletion (European Environment Agency 2003; Frederick et al. 2000). A number of studies have been carried out to study whether this increased UV-B radiation has a significant impact on plants (Nasser 2003; Gao et al. 2003). Plant responses to increased UV-B radiation include a reduction in growth and biomass, and in the area and thickness of leaves, as well as an increase in reflective surface waxes and amount of UV-B absorbing compounds. Direct effects on stomata conductance, photosynthetic rate, photosynthetic apparatus and the amount of photosynthetic pigments are reported as well (Ambasht, Agrawal 2003; Gonzalez et al. 1996). Plant sensitivity to UV-B is species specific and some species are extremely tolerant while some are very sensitive (Juknys et al. 2008; Wang et al. 2007).

Another problem related to ozone is increasing tropospheric ozone concentration (Krupa 2003). Troposphere ozone is a secondary pollutant formed in the sunlight from precursors such as nitrogen dioxide in the presence of volatile organic compounds. Concentrations of ozone have risen by 1%-2% per year during the last several decades and nearly one-quarter of the Earth surface is at the risk from ozone impact. Vegetation is increasingly affected by this environmental stressor (Morgan et al. 2003). Significant increases in the concentration of ground level ozone were detected in Lithuania during the last 20 years (Girgždienė, Girgždys 2003).

Experiments have shown that increased tropospheric ozone concentration induces negative impacts on growth and photosynthetic processes (Skärby 1994; Küppers et al. 1994; Miller et al. 1994). A number of reports deal with visible injury to plants caused by ozone, since high levels of ozone may cause chlorotic and necrotic lesions depending on concentration and length of exposure (Rao et al. 2000; Wu, Von Tiedemann 2004).

Ozone, as one of the most powerful agents of oxidative stress, forms free radicals and may result in a wide range of physiological changes, such as alteration of membranes, reduction in the amount and activity of Rubisco, and acceleration of leaf senescence (Baier et al. 2005; Gielen et al. 2007; Ludwikow et al. 2004). Many researchers have shown that ozone can reduce chlorophyll and carotenoid content in leaves (Morgan et al. 2003; Saitanis et al. 2001; Sakalauskaitė ir kt. 2006). However, plant sensitivity to the impacts of ozone is species or even cultivar specific (Juozaitytė et al. 2007; Kline et al. 2008). The aim of this study is to compare the tolerance of different spring barley (*Hordeum vulgare* L.) cultivars to the impacts of UV-B radiation and ozone exposure.

2. Materials and Methods

Seven spring barley (*Hordeum vulgare* L.) cultivars (‘Annabell’, ‘Henni’, ‘Scarlet’, ‘Barke’, ‘Jersey’, ‘Tolar’ and ‘Aura’) were selected for the study. Experiments were conducted under controlled environmental conditions in the growth chambers at a temperature regime of 21/17^{o}C (day/night) and a 16-h photoperiod. Plants were grown in pots containing pH 6.0-6.5 peat substrate, 25 plants per pot. All treatments were run in three replicates. Seven-day-old plants were divided into two halves. One half of the plants for each barley cultivar was transferred into identical growth chambers maintained under the same environmental conditions but irradiated with UV-B radiation or exposed to ozone. UV-B radiation was provided by fluorescent tubes TL 40W/12RS UV-B Medical, Philips and was measured with a VLX-3 radiometer (Vilber-Lourmat, France). Plants were irradiated with 8 kJ m^{-2} d^{-1} UV-B dose for six days.

Ozone was generated by an OSR-8 (Ozone Solutions, Inc.) and monitored with a portable ozone sensor OMC-1108 (Ozone Solutions, Inc.). Plants were exposed to 240 µg m^{-3} ozone concentration for seven hours per day. The reference treatment plants were maintained with zero UV-B doses and no ozone.

Dry above-ground biomass and concentration of photosynthetic pigments (chlorophylls *a, b* and carotenoids) were measured at the end of each treatment. The biomass samples were dried to constant weight in an oven at 70°C. Photosynthetic pigments were analysed using a spectrophotometer (Genesys 6, ThermoSpectronic, USA) after extracting in 100% acetone the extracts prepared according to Wettstein’s method (Wettstein 1957).

The independent-samples *t*-test was applied to estimate the significance of the differences between reference and treatment values. All analyses were performed using the STATISTICA statistical package and results were expressed as mean values and their standard errors.

3. Results and discussion

UV-B radiation caused rather different reductions in dry biomass of treated spring barley cultivars (Fig. 1). Lithuanian spring barley cultivar ‘Aura’ and foreign cultivars ‘Barke’, ‘Jersey’ and ‘Tolar’ were the most susceptible to UV-B radiation. After six days exposure to UV-B radiation (8 kJ m^{-2}), dry biomass of ‘Aura’ was reduced by 57%, while ‘Barke’, ‘Jersey’ and ‘Tolar’ were reduced by approximately 45% as compared to the reference treatment. ‘Scarlet’ and ‘Henni’ cultivars were the most tolerant to UV-B radiation and their biomass decreased only by ~ 30% ($p<0.05$).

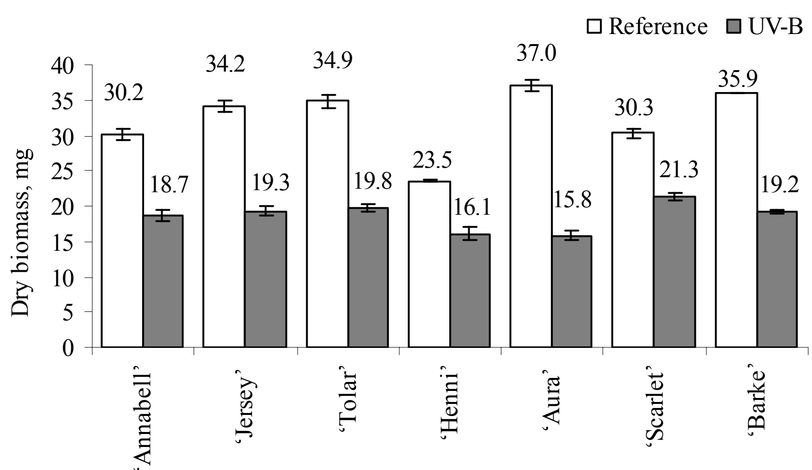

Fig. 1. Impact of UV-B radiation on dry biomass of different spring barley cultivars

The chlorophyll (*a+b*) content in leaves decreased for nearly all analysed cultivars under exposure to UV-B radiation (Table 1). The largest reduction in the content of chlorophyll was detected for cultivars ‘Barke’ and ‘Tolar’ – 35%, while cultivars ‘Annabell’ and ‘Aura’ – decreased approximately by 30%. The least and statistically insignificant losses in the content of chlorophyll (3.5%) were characteristic of ‘Henni’ cultivar, whereas a small increase (4%) in the content of chlorophyll was detected in the leaves of cultivar ‘Jersey’.

Table 1. Impact of UV-B radiation on photosynthetic pigments in the leaves of different spring barley cultivars

Cultivars	Treatment	Chlorophyll a+b content mg g^{-1}	Chlorophyll a/b ratio	Carotenoids content mg g^{-1}
'Annabell'	Reference	1.39* ± 0.020	2.89* ± 0.098	0.31* ± 0.027
	UV-B	0.99* ± 0.012	2.03* ± 0.032	0.42* ± 0.001
'Jersey'	Reference	1.22* ± 0.011	3.40 ± 0.141	0.27* ± 0.004
	UV-B	1.27* ± 0.010	3.25 ± 0.087	0.51* ± 0.010
'Tolar'	Reference	1.43* ± 0.017	3.35 ± 0.223	0.33* ± 0.008
	UV-B	0.93* ± 0.003	3.22 ± 0.029	0.47* ± 0.001
'Henni'	Reference	1.15 ± 0.029	3.66* ± 0.457	0.27* ± 0.004
	UV-B	1.11 ± 0.010	2.67* ± 0.127	0.32* ± 0.003
'Aura'	Reference	1.48* ± 0.007	3.38* ± 0.140	0.34* ± 0.014
	UV-B	1.06* ± 0.017	2.61* ± 0.089	0.54* ± 0.006
'Scarlet'	Reference	1.38* ± 0.012	3.47* ± 0.087	0.31* ± 0.001
	UV-B	1.16* ± 0.016	2.89* ± 0.126	0.43* ± 0.005
'Barke'	Reference	1.33* ± 0.008	3.26* ± 0.023	0.30* ± 0.001
	UV-B	0.86* ± 0.008	3.12* ± 0.019	0.39* ± 0.004

** - significant difference, $p<0.05$*

The ratio of chlorophyll *a/b* has decreased in the leaves of all analysed cultivars (Table 1) and it is considered as an evidence that chlorophyll *a* is more sensitive to the impact of UV-B radiation than chlorophyll *b*. As it seen in Table 1, the largest reductions in the chlorophyll *a/b* ratio were found in cultivars 'Annabell' and 'Henni', and minimal changes in this indicator were detected in 'Tolar' and 'Barke' cultivars.

The content of carotenoids in the leaves of these spring barley cultivars increased after exposure to UV-B radiation The greatest increase in content of carotenoids was detected for 'Jersey' (88.9%) and 'Aura' (58.8%) cultivars and the least was found in 'Henni' (18.5%) cultivar.

Generalizing the changes in different analysed indicators, cultivars 'Aura', 'Barke' and 'Tolar' could be considered as the most sensitive, whereas cultivars 'Scarlet', 'Jersey' and 'Henni' as the most tolerant to the impact of UV-B radiation.

Under ozone exposure (240 µg m^{-3}) the dry biomass of all analysed barley cultivars decreased from 19 to 39% (Fig. 2). The least changes in biomass as compared to reference treatment (zero ozone concentration) were observed in 'Aura' (19%) and 'Scarlet' (23%) cultivars and the greatest changes were observed in 'Barke' (36%) and 'Annabell' (39%) cultivars.

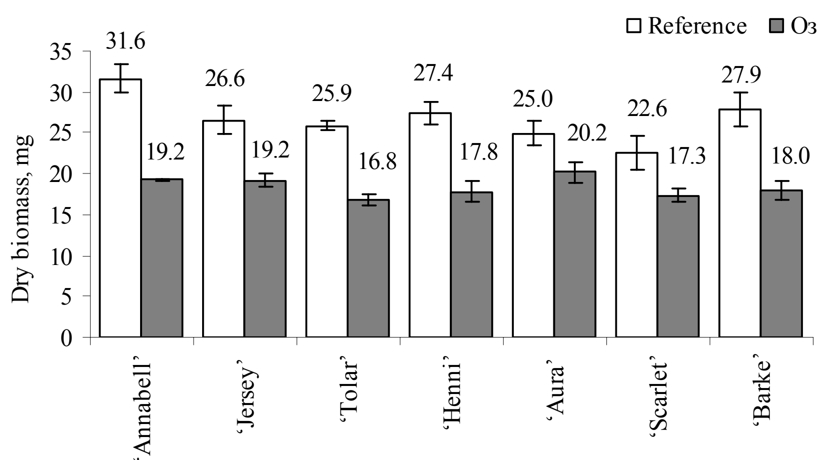

Fig. 2. Impact of ozone on dry biomass of different spring barley cultivars

The total content of chlorophyll (*a+b*) decreased under exposure to ozone (Table 2). The least losses of chlorophyll (31%) were detected in 'Aura' cultivar whereas the content of chlorophyll in 'Henni' leaves decreased by 44% and in 'Scarlet' leaves by 52% as compared to the reference treatment.

The ratio of chlorophyll *a/b* decreased statistically significantly ($p<0.05$) in the leaves of most of the analysed cultivars (Table 2). The largest reduction in the chlorophyll *a/b* ratio under ozone exposure was detected in 'Barke' and 'Jersey' cultivars, and no changes were detected in 'Tolar' and 'Annabell' cultivars (Table 2).

Table 2. Impact of ozone on photosynthetic pigments in the leaves of different spring barley cultivars

Cultivars	Treatment	Chlorophyll *a+b* content mg g^{-1}	Chlorophyll *a/b* ratio	Carotenoids content mg g^{-1}
'Annabell'	Reference	2.29* ± 0.823	2.54 ± 0.727	0.38* ± 0.008
	O_3	0.97* ± 0.026	3.06 ± 0.147	0.27* ± 0.015
'Jersey'	Reference	1.63 ± 0.099	3.36* ± 0.051	0.40 ± 0.020
	O_3	1.08 ± 0.010	2.25* ± 0.087	0.32 ± 0.010
'Tolar'	Reference	1.55* ± 0.039	3.35 ± 0.094	0.40* ± 0.006
	O_3	0.92* ± 0.083	3.24 ± 0.105	0.27* ± 0.031
'Henni'	Reference	1.51* ± 0.119	3.24* ± 0.099	0.37* ± 0.019
	O_3	0.84* ± 0.057	2.54* ± 0.124	0.22* ±0.006
'Aura'	Reference	1.41 ± 0.075	3.18 ± 0.120	0.41* ± 0.012
	O_3	0.98 ± 0.061	2.93 ± 0.141	0.27* ± 0.005
'Scarlet'	Reference	1.58*± 0.138	2.80 ± 0.074	0.39 ± 0.028
	O_3	0.76* ± 0.161	2.33 ± 0.369	0.26 ± 0.052
'Barke'	Reference	1.59* ± 0.056	3.27* ± 0.078	0.38* ± 0.008
	O_3	0.92* ± 0.104	2.50* ± 0.185	0.24* ± 0.011

** - significant difference, $p<0.05$*

Data presented in Table 2 show that the content of carotenoids in the leaves of all analysed cultivars, contrary to the case under UV-B irradiation, decreased under ozone exposure. The content of carotenoids in 'Jersey', 'Annabell' and 'Scarlet' leaves decreased by 20%, 29% and 33%, respectively. The greatest losses in the content of carotenoids were detected in 'Henni' cultivar - 41%.

Taking into consideration changes in different analysed indicators, cultivars 'Aura', 'Jersey' and 'Tolar' can be considered most tolerant and cultivars 'Scarlet', 'Annabell' and 'Henni' as most sensitive to the impact of ozone.

This comparison of the impacts of UV-B radiation and ozone on various spring barley cultivars has shown that the reaction of these cultivars to two different stressors differs greatly. In most cases the cultivars which are tolerant to the impact of ozone are sensitive to UV-B and vice versa – cultivars which are tolerant to UV-B are sensitive to the impact of ozone.

Cultivars 'Scarlet', 'Annabell' and 'Henni' were found to be most sensitive to the impact of ozone and while cultivars 'Aura', 'Barke' and 'Tolar' were most sensitive to UV-B radiation. On the other hand, cultivars 'Aura', 'Jersey' and 'Tolar' were the most tolerant to the impact of ozone while cultivars 'Scarlet', 'Jersey' and 'Henni' were the most tolerant to the impact of UV-B.

As has been shown by different authors, the targets of UV-B radiation and ozone in plant cells are very different. Elevated UV-B doses have a detrimental impact on the photosynthetic apparatus, they break down disulfide bridges in protein molecules, do DNA damage, bring about changes in gene expression, alter activity of enzymes and the content of protective pigments (Qaderi 2007). At the same time, ozone, as one of the most powerful agents of oxidative stress, forms free radicals and may result in a wide range of physiological changes such as peroxidation of the plasmalemma and other membranes, reduction in the amount and activity of Rubisco and loss of chlorophyll (Fuhrer 2003).

Despite significant differences in damaging mechanisms of UV-B and ozone, a reduction in chlorophyll content was a common feature of the impact of both analysed stressors (Tables 1 and 2). Data on the reduced chlorophyll content and inhibition of growth (biomass formation) under the impact of UV-B radiation and ozone exposure were previously reported by different researchers (Ambasht, Agrawal 2003; Ariyaphanphitak et al. 2002; Pilipavičius et al. 2006). Both analysed stressors have resulted in a decrease of the chlorophyll *a/b* ratio and chlorophyll *a* is likely to be more sensitive to the impact of both stressors than chlorophyll *b*.

Carotenoids not only serve as accessory pigments for harvesting photosynthetically active radiation in the region of wavelengths not covered by chlorophylls, but they also play a significant role as efficient quenchers of high energy short-wave (UV) radiation and they are able to reduce photooxidative stress (Bartley, Scolnik 1995; Fedina et al. 2003). Our research has shown very different reaction of carotenoids to the impact of the two analysed stressors. An essential increase in the content of carotenoids was registered under UV-B radiation while ozone, in contrast, resulted in a decrease in the carotenoid content. According to other authors the content of carotenoids is usually less reduced than chlorophylls, moreover it even increases under an impact of UV-B or other stressors (Fedina et al. 2003; Garcia-Plazaola, Becerril 2000).

4. Conclusions

The impact of UV-B radiation and ozone on various spring barley cultivars differs greatly and in most cases the cultivars which are tolerant to the impact of ozone are sensitive to UV-B and vice versa

– cultivars which are tolerant to UV-B radiation are sensitive to the impact of ozone.

Both analysed stressors have resulted in a more pronounced decrease in the content of chlorophyll *a* than chlorophyll *b* and a decrease in the chlorophyll *a/b* ratio in the leaves of these different spring barley cultivars. This is a common feature of both the impact of UV-B radiation and the ozone exposure.

Interestingly, the reaction of carotenoids to the two stressors is absolutely different. An increase in the content of carotenoids has been found under UV-B radiation and it could be considered as a defence reaction against high energy short-wave radiation. In contrast, the impact of ozone has resulted in a decrease in carotenoids.

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Ultravioletinės (UV-B) spinduliuotės ir ozono poveikio skirtingoms vasarinių miežių veislėms tyrimai

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Kontroliuojamomis aplinkos sąlygomis tirtas ultravioletinės (UV-B) spinduliuotės (8 kJ m–2 d–1) ir ozono (240 µg m–3) poveikis septynioms vasarinių miežių (Hordeum vulgare L.) veislėms. Tirtų miežių veislių jautrumas ultravioletinės spinduliuotės poveikiui labai skyrėsi nuo jų jautrumo ozono poveikiui. Daugeliu atvejų veislės, pakančios UV-B spinduliuotei, buvo jautrios ozono poveikiui ir atvirkščiai. Nustatyta, kad abu tirti stresoriai slopino chlorofilo sintezę, chlorofilo a koncentracija sumažėjo labiau nei chlorofilo b. Taip pat sumažėjo chlorofilo a ir chlorofilo b santykis. Dėl UV-B spinduliuotės karotinoidų koncentracija lapuose padidėjo, dėl ozono poveikio, priešingai, karotinoidų koncentracija visų tirtų veislių miežių lapuose sumažėjo.