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Event: XV International Conference on Pulsed Lasers and Laser Applications, 2021, Tomsk, Russian Federation

Physiological action of UVB radiation on wheat sprouts (*Triticum aestivum* L.)

Eduard A. Sosnin[*a,b], Anastasia A. Burenina[c], Elena N. Surnina[c], Yulia Yu. Fadeeva[b], Tatiana P. Astaphyrova[c]

[a]Institute of High Current Electronics, Siberian Branch of Russian Academy of Science, 2/3 Akademicheski ave., Tomsk, 634055, Russia
[b]Tomsk State University, 36 Lenina st., 634050, Tomsk, Russia
[c]Botanic garden of Tomsk State University, 36 Lenina st., 634050, Tomsk, Russia

ABSTRACT

This work continues the cycle of our research on the physiological effect of UVB radiation on plants. The physiological effect of UVB radiation (308 nm, doses of 0.5, 1.4 and 2.7 J/cm^2) on the structural and functional characteristics of wheat seedlings was determined. With an increase in the radiation dose, a decrease in plant height, leaf area and the amount of photosynthetic pigments was revealed, photosynthesis parameters decreased, and the content of low-molecular-weight antioxidants increased. This means that UVB priming of wheat sprouts should be carried out at even lower doses than were selected in the experiments.

Keywords: excilamp, priming, sprouts, *Triticum aestivum* L., ultraviolet radiation.

1. INTRODUCTION

Solar radiation is the most important physical factor that determines the life of plants. Photosynthetically active radiation (FAR) plays a key role in the formation of yield, including blue 400-500 nm, green 500-600 nm and red 600-700 nm visible light ranges. It penetrates through the atmosphere and reaches the surface of the Earth. Therefore, the use of FAR artificial sources has become common in agricultural practice.

The role of solar ultraviolet (UV) radiation is not so unambiguous. Most often, the ultraviolet range is divided into three sections: UVC or "hard ultraviolet" ($200 < \lambda < 280$ nm), UVB ($280 < \lambda < 320$ nm), UVA or "soft ultraviolet" ($320 < \lambda < 400$ nm). Only UVA and part of UVB radiation reaches the Earth's surface.

Soft ultraviolet light is still detected by specialized receptors of the leaf apparatus of plants. Increased doses of UVA irradiation reduce the leaf area per unit of plant biomass, but increase the productivity of biomass, including nitrogen content, and are also able to accelerate upswelling of seeds and enhance respiration and growth processes[1,2]. It is noted that the stimulating effect of the study on germination is manifested only in seeds of a reduced degree of quality that produce sprouts with morphological defects.

Previously, we have convincingly shown[3-7] that the treatment of seeds of various plants with UVB radiation *subdoses* is an environmentally safe method of increasing plant productivity and yield, as well as protecting plants from various stresses. Our results are in agreement with the data on the so-called UV priming[8].

Due to the small exposures of UVB radiation in the natural habitat of plants, we initially conjectured that it was of a signal nature. Starting our research more than 15 years ago, we also assumed that the effect of UVB radiation in natural conditions is carried out seasonally, mainly during the growing season of plants. Therefore, UVB subdoses should have a positive effect only on seeds and seedlings of plants. Currently, the positive effect on seeds is proven, but the effect on seedlings is not established. Therefore, this work will be devoted to identifying changes in the structural, functional and biochemical parameters of wheat seedlings after exposure to UVB radiation.

*badik@loi.hcei.tsc.ru; phone 8(3822)900-207; fax 8(3822)492-410; www.hcei.tsc.ru

XV International Conference on Pulsed Lasers and Laser Applications, edited by Anton V. Klimkin, Maxim V. Trigub, Victor F. Tarasenko, Proc. of SPIE Vol. 12086, 120861E · © 2021 SPIE · 0277-786X · doi: 10.1117/12.2605104

2. MATERIALS AND METHODS

The object of the study was 6-day-old sprouts of spring wheat (*Triticum aestivum* L.) of the «Iren» cultivar. In the experiment, selected seeds of the 2019 harvest were used, the germination of which was at least 95 %. Wheat seeds of 40 pieces in four replications were placed in plastic containers with sand. The sand was pre-calcined in a drying cabinet at a temperature of 200 °C, then moistened by 60% of the total moisture capacity. For the first three days, the containers with seeds were kept in the thermostat TSO-1/18 SPU (Russia) at a temperature of 20 °C. On day 4, the sprouts were treated with XeCl-excilamp radiation (308 nm) for 120, 360 and 600 s, which corresponded to energy exposures (doses) of 0.5, 1.4 and 2.7 J/cm^2, then the containers were placed in a LAB-LINE climate chamber (Czech Republic) and the plants were cultivated at a 16-hour photoperiod at a temperature of 20-22 °C.

As in our previous works, the choice of the XeCl-excilamp as a radiation source was because it is an artificial simulator of the edge of solar UVB radiation spectra that is transmitted by the atmosphere. This exciplex lamp (model XeCl_BD_P) provides an intense radiation band in the wavelength range $\lambda \sim 290$-320 nm with a maximum radiation at $\lambda = 308$ nm and a half-width band $\Delta\lambda_{1/2} = 1.9$ nm.

To assess the influence of irradiation parameters on the processes of plant vegetation, morphometric parameters were evaluated in all samples (length of the root system, length of seedlings, mass of the root and aerial part of plant). The leaf area was determined using computer scanning in the ImageJ software. To determine the dry mass, the sprouts were drained in a desiccator at 70° C to a constant mass. The specific surface area of the leaves (SSAL) was calculated as the ratio of the dry mass of the leaves to their area.

The amount of chlorophylls a and b, as well as the amount of carotenoids, were determined in ethyl alcohol using a UV-1650 spectrophotometer Shimadzu (Japan) at wavelengths of 665, 649 and 440.5 nm. The content of ascorbic acid was determined according to the method[9], the content of free proline in the leaves was determined using an acidic ninhydrin reagent according to the method[10].

The anatomical structure of wheat leaves was studied using well-known methods[11], and the stomatal index was calculated according to the formula of A. Kestner[12].

Photos of micro-preparations of leaves and microscopic measurements were made using a Carl Zeiss Axio Lab light microscope with the AxioCam ERc 5s digital camera using the Axio Vision 4.8 program. The measurement results were processed statistically according to the method of G.N. Zaitsev[13] using the Statistica 8.0 program. The following indicators were determined: M – arithmetic mean, m – arithmetic mean error, CV – coefficient of variation. Anatomical indicators are considered to be low-variable if CV < 20%, medium-variable - at CV = 20–40% and highly variable at CV > 40%[14]. When assessing the reliability of differences in independent samples, the value of the *t*-test statistics calculated under the assumption of equal variances in the samples; the value of the *t*-test statistics calculated under the assumption of differences in variances in the samples; the value of the Fisher *F*-test statistics were evaluated. Valuable differences were determined at the significance level $p < 0.05$.

3. RESULTS AND DISCUSSION

Studies conducted with wheat sprouts after treatment with XeCl-excilamp showed that they differed in a number of morphological parameters. The height of the shoots of the experimental groups was 5.8–9.5% less compared to the control ones, wheat plants became more compact, which was confirmed by a significant increase in the ratio of the root length to the shoot (see Table 1). At the same time, the area of wheat plumule sheath decreased in all groups with XeCl-excilamp treatment by 10-14% compared to the control. These data are consistent with the common mechanisms of adaptation to UVB radiation at the level of the whole plant by reducing the height of plants and increasing the number of internodes with enhanced branching[15].

The irradiation level affected the accumulation of biomass of wheat sprouts. The root mass increased relative to the control in all irradiation groups by 20-22% with the mass of the aboveground part unchanged (Tables 2, 3). What is the reason for this? Several decades ago, it was discovered that the root system has the same photoreceptors as the aboveground parts of plants, and perhaps this allows the roots to respond to the direction, intensity and wavelength of light[16,17]. In our experiment, an increase in mass at a constant length may indicate a UVB-mediated increase in the thickness of wheat roots.

An important parameter of the leaf apparatus that characterizes the vital activity of plants is the specific surface area of the leaves (SSAL). In the groups after treatment with XeCl-excilamp, the SSAL was higher than in the control by 13-14% (Table 1). We assume that the increased value of the SSAL of the leaves of the experimental groups is most likely due to a decrease in the leaf area.

Table 1. Growth parameters of wheat seedlings after treatment with XeCl-excilamp radiation

Group	**Root length, cm**	**Sprout height, cm**	**Root length / Sprout height**	**Assimilating surface, см2**	**SSAL, µg/cm2**
Control	12.84 ± 0.32	19.15 ± 0.29	0.67 ± 0.02	3.58 ± 0.09	3.11 ± 0.10
0.5 J/cm^2	12.95 ± 0.23	17.45 ± 0.29*	0.75 ± 0.02*	3.23 ± 0.09*	3.52 ± 0.12*
1.4 J/cm^2	12.64 ± 0.36	17.34 ± 0.32*	0.73 ± 0.01*	3.09 ± 0.10*	3.56 ± 0.12*
2.7 J/cm^2	12.71 ± 0.28	18.04 ± 0.30*	0.73 ± 0.02*	3.10 ± 0.10*	3.56 ± 0.10*
Here and further, the '*' sign marks significant differences between control and experience at $p < 0.05$.					

Table 2. Action of UV radiation on the wet mass of wheat seedlings

Group	**Wet weight, mg**			**Root / Herb**
	Root	**Herb**	**Leaf**	
Control	142.67 ± 4.38	126.29 ± 3.56	79.43 ± 2.11	1.14 ± 0.04
0.5 J/cm^2	173.55 ± 8.03*	117.90 ± 3.31	74.20 ± 2.39	1.47 ± 0.06*
1.4 J/cm^2	171.31 ± 7.85*	116.74 ± 3.33	74.56 ± 2.09	1.49 ± 0.09*
2.7 J/cm^2	171.35 ± 5.58*	116.70 ± 3.65	74.75 ± 2.69	1.48 ± 0.05*

Table 3. Action of UV radiation on the air-dry mass of wheat seedlings

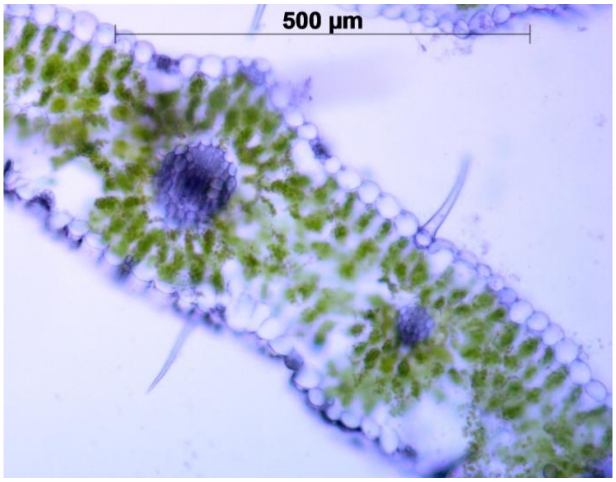
Group	**Dry weight, mg**			**Root / Herb**
	Root	**Herb**	**Leaf**	
Control	28.29 ± 1.69	18.14 ± 0.51	11.48 ± 0.38	1.56 ± 0.09
0.5 J/cm^2	33.55 ± 1.47*	17.35 ± 0.47	11.20 ± 0.43	1.95 ± 0.11*
1.4 J/cm^2	33.17 ± 1.62*	17.20 ± 0.59	10.95 ± 0.35	1.92 ± 0.10*
2.7 J/cm^2	33.11 ± 1.53*	16.82 ± 0.44	10.55 ± 0.44	1.95 ± 0.09*

Table 4. Action of XeCl-excilamp radiation on the content of photosynthetic pigments of the first wheat leaf, µg/g of wet mass

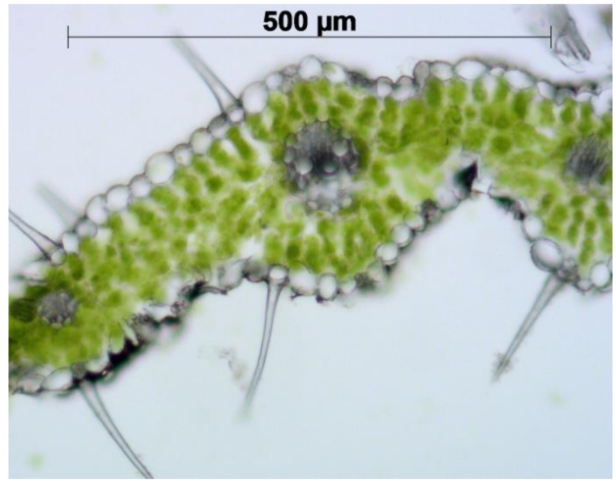
Group	**Chlorophyll**		***a* / *b***	**Carotenoids sum (cs)**	**(*a*+*b*) / cs**
	a	***b***			
Control	1146.45 ± 12.03	370.58 ± 4.38	3.1	298.10 ± 7.64	5.1
0.5 J/cm^2	1138.34 ± 30.80	339.88 ± 6.39*	3.4*	289.93 ± 8.43	5.1
1.4 J/cm^2	1034.42 ± 4.13*	314.09 ± 4.48*	3.3*	267.36 ± 3.04*	5.0
2.7 J/cm^2	957.59 ± 10.73*	296.22 ± 4.63*	3.2	248.18 ± 6.07*	5.1

Twenty standard characteristics of the anatomical structure of the leaves of wheat sprouts subjected to a single treatment with UVB radiation of XeCl-excilamp were studied. Among them, we can note an increase in trichomes density. It increased on the upper epidermis at an irradiation dose of 0.5 J/cm^2 by 2.8 times and on the lower – by 2 times, increasing with radiation dose, which was also visually noticeable (Figure 1).

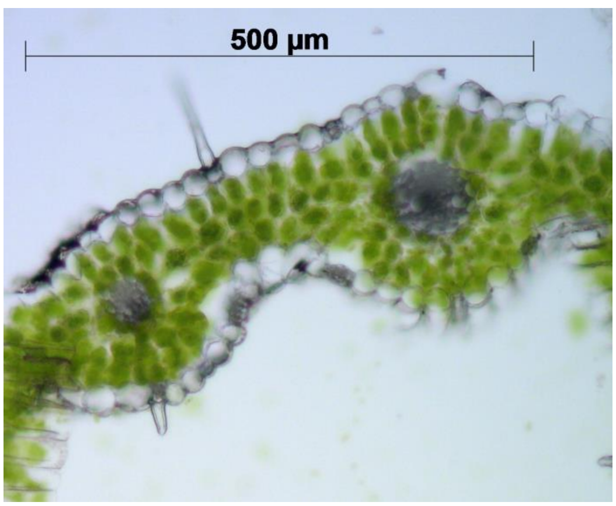
After treatment with XeCl-excilamp, the amount of chlorophyll *a* decreased with an increase in the radiation dose (1.4 and 2.7 J/cm^2) by 10 and 16%, respectively, compared with the control (Table 4). Chlorophyll *b* was more sensitive to radiation, its amount decreased by 8% already at an irradiation dose of 0.5 J/cm^2.



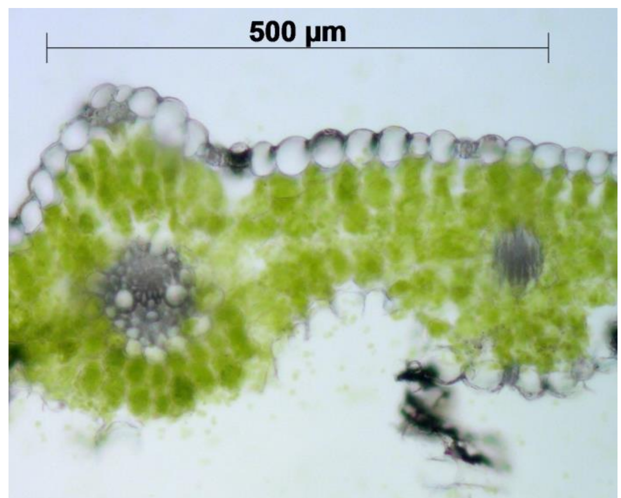
Control



0.5 J/cm^2



1.4 J/cm^2



2.7 J/cm^2

Figure 1. Cross-section cut of a leaf of the studied samples of wheat seedlings

The content of carotenoids after UVB treatment decreased at radiation doses of 1.4 and 2.7 J/cm^2, apparently due to their degradation, since these pigments are components of the antioxidant protection system.

An equally important role in characterizing of photosynthetic apparatus functioning is played by the ratio of the amount of chlorophylls to carotenoids. Normally, this ratio is stable, but it actively reacts to changes in various environmental factors. The treatment of wheat sprouts with XeCl-excilamp did not affect this indicator, which may indicate the stability of the light-collecting function of the pigment complex under the influence of adverse conditions.

The study of the integral functional characteristics of the leaf apparatus (photosynthesis and transpiration) also revealed the dependence of these indicators on the radiation dose. In our study, there was a decrease of photosynthesis intensity under the influence of XeCl-excilamp treatment at doses of 1.4 and 2.7 J/cm^2 by 13 and 19%, respectively. This is consistent with other studies confirming that an increase in the dose of UVB radiation can lead to a 3-90% decrease in photosynthesis in plants due to direct (effect on the photosystem) and indirect (decrease in the content of pigments and leaf area) effects[18].

The transpiration intensity decreased at an irradiation dose of 2.7 J/cm^2 by 9% relative to the control. The obtained data may indicate that the treatment of sprouts with an excilamp was decisive for the parameters of photosynthesis (the content of pigments and photosynthesis intensity) and to a lesser extent affected the water exchange of wheat seedlings.

In addition, the measurements revealed: 1) an increase in the content of flavonoids in the leaves of wheat sprouts by 13-15% at all doses of excilamp irradiation; 2) a significant increase in the content of proline by 22% at an irradiation dose of 1.4 J/cm^2, and a 20% decrease at a dose of 2.7 J/cm^2; 3) an increase in the amount of ascorbic acid in wheat leaves at irradiation doses of 1.4 and 2.7 J/cm^2 by 12-14% compared to the control. All these indicators indicate that the plant is protected in response to stress.

4. CONCLUSION

The physiological effect of UVB radiation (308 nm, doses of 0.5, 1.4 and 2.7 J/cm^2) on the structural and functional characteristics of wheat sprouts was determined. At all doses the height of plants and the area of the assimilating surface decreased, the specific surface area of the leaves and the mass of the root increased. After irradiation of seedlings, a decrease in the number of stomata was noted with an increase in their size, an increase in the density of leaf trichomes, an increase in the thickness of the upper epidermis (differences affecting the epidermis and its elements performing protective functions). No significant differences were found in the structure of the mesophyll of the leaf and the conducting system.

With an increase of UVB radiation dose a decrease in the parameters of photosynthesis was observed both due to a direct effect on the photosystem, and due to a decrease in the content of pigments and the area of leaves. Different radiation doses also affected the synthesis of low molecular weight antioxidants – flavonoids, proline and ascorbic acid. Their content in the leaves increased, especially at doses of 1.4 and 2.7 J/cm^2.

Based on the data obtained, it can be concluded that at an irradiation dose of 0.5 J/cm^2, plants are able to adapt to the effects of UVB radiation. The degree of their damage, determined by the content of proline, ascorbic acid and chlorophylls *a* and *b*, is insignificant. It is recommended to use a dose of less than 0.5 J / cm^2 for UVB-priming of wheat seedlings.

ACKNOWLEDGMENTS

The study was carried out within the framework of project No. 8.1.29.2018 (Tomsk State University competitiveness improvement program) and within the framework of the State Task of the IHCE SB RAS, project No. FWRM-2021-0014.

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