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AGE-DEPENDENT MORPHOPHYSIOLOGICAL CHANGES AND BIOCHEMICAL COMPOSITION OF *Lactuca sativa* L. PLANTS INFLUENCED BY Se AND SOLAR RADIATION OF VARYING INTENSITY

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Abstract

Selenium, a micronutrient significantly involved in plant metabolism control, is also essential for human. Se regulates plant growth and protects plants from many adverse factors. The relevance of the issue is particularly high in biogeochemical provinces with selenium deficiency. Improvement of cultivation of greenhouse crops is also largely associated with optimization of the light regime. In this paper, we first reported on how selenite and selenate ions, in combination with intensity of UV-A + PAR, impact on growth and age-associated accumulation of primary and secondary metabolites in *Lactuca sativa* L. plants. These results will contribute to a better understanding of signaling elements involved in metabolic regulation. Prior to sowing, the seeds were treated with 4 % sodium selenite or sodium selenate in test and with water in control. Light intensity and spectral characteristics were changed by covering a greenhouse with polyethylene films F1 and F2 (for F2, the UV-A transmission was 40-50 % higher and PAR was 30-35 % higher as compared to F1). More insolation under F2 led to elevated content of leaf chlorophyll a (Chla), chlorophyll b (Chlb), and sugars in 60-day-old plants, thus promoting shoot development due to formation of more internodes (by 15 %) and higher stem weight compared to F1 ($p < 0.05$). Se + F1 intensified accumulation of carbohydrates and proteins, increased leaf area and caused the decline in ascorbic acid content, while F2 stimulated accumulation of ascorbic acid and flavonoids. Higher accumulation of leaf pigments (carotenoids, flavonoids and anthocyanins), lower carbohydrates in juvenile leaves under F2, and a greater number of leaf layers on a stem, due to synergic effect of light and Se, were peculiar of selenate action. Selenite + F1 led to higher content of carotenoids in juvenile leaves, whereas under selenite + F2 the level of ascorbic acid and flavonoids was higher in aging leaves. The highest content of reducing sugars (RS) and soluble proteins was in the mature leaves (layers 8 to 16) of control plants (F1). When solar radiation going up, a rise of RS level by 30 %, 45 % and 2.3 times occurred in aging leaves (layers 4-7), in adult leaves, and in young leaves (layers 17-21), respectively, while the protein content decreased in aging leaves ($p < 0.05$). Both SeO_3^{2-} and SeO_4^{2-} resulted in a higher level of RS and proteins in young leaves and kept this high in aging ones. The young and aging leaves of the control F1 plants differed in the content of flavonoids (Fla) 6-fold. Both selenium ions reduced the Fla level in mature and aging leaves by 20-30 % ($p < 0.05$), and SeO_4^{2-} led to a 4-fold increase in Fla of young leaves (F1). Increasing solar radiation (F2) resulted in the decline of Fla content. SeO_3^{2-} provided a higher Fla level in aging and mature leaves, whereas SeO_4^{2-} enhanced Fla accumulation in young leaves. At a higher light intensity, SeO_4^{2-} + F2 increased the carotenoids content by 76 %, while SeO_3^{2-} + F1 ensured only a 60 % increase ($p < 0.05$). In increasing insolation, both selenium ions elevated the shoot dry weight and the content of low molecular antioxidants (ascorbic acid and Fla) in plants. Thus, our findings showed the dependence of plant growth and metabolism on specific forms of selenium under varying intensity of solar radiation. These biomarkers should be accounted while growing plants using selenium in different lighting conditions.

Keywords: *Lactuca sativa* L., sodium selenite, sodium selenate, solar radiation, carotenoids,

reducing sugars, proteins, ascorbic acid, flavonoids, anthocyanins, primary and secondary metabolites

Selenium (Se) is an essential element for animals and humans, as well as a regulator of biochemical processes in plants. In some countries, such as China, Egypt, and Thailand, its concentration is reduced [1]. In Russia, the most selenium-deficient soils are located in Buryatia, the Chita Region, and the Khabarovsk Territory [2], where the minimal content of selenium in wheat grains is recorded. Selenium deficiency in the human diet leads to endemic osteopathy, myxomatous endemic cretinism, development of cardiological and oncological diseases, pathologies of the reproduction system, malfunctioning of the thyroid and pancreatic glands, which is related to abnormalities in synthesis and functioning of 25 selenium-dependent proteins [3, 4]. Agricultural plants are biofortified through foliar or soil application of Se compounds [5]. Se regulates plant growth, modifies the carbohydrate composition and increases resistance to abiotic stresses induced by cold, drought, UV-B rays, water shortage, salinity, and heavy metals [1, 6-8]. The positive effects of Se depend on its dose and the plant genotype and are accompanied by the activation of antioxidant protection in the cells [5, 8, 9]. The existence of several inorganic forms of Se including selenites (SeO_3^2) and selenates (SeO_4^2) raises the question about their functions and availability to plants, the impact on the productivity and sustainability of crops to the effects of light.

Light plays an important role in the regulation of plant life. It activates signaling pathways, which are controlled by selective sensory pigments and are involved in the implementation of growth and metabolic processes. Selective light alters plant growth and the amount of absorbed Se [10]. The total efficiency of the mixed stream of solar radiation on the productivity of crops in greenhouses is less studied [11]. It is known that removing UV(A + B) (λ= 280-400 nm) from a stream of solar radiation enhances the growth of terrestrial and subterranean plant organs, increases the concentration of photosynthetic pigments, photosynthetic enzyme activity and efficiency of the photosynthetic systems PSII [12]. At the same time, it is suggested that the role of photosynthetically active radiation (PAR λ = 400-700 nm) is to modify the sensitivity and photo-morphogenetic responses of plants to UV-B radiation (λ = 280-320 nm). Increasing the ratios PAR/UV-B and UV-A/UV-B is important for reducing the damage from UV-B to terrestrial and aquatic plants [13]. However, there are few studies on the interaction of UV-A (A = 320-400 nm) and PAR.

The authors have demonstrated for the first time significant differences in the manifestation of responses to the combined action of selenium and light between lettuce leaves of different ages characterized by the varying intensity of growth processes.

The aim of this work was to examine the effects of different forms of selenium (SeO_3^{2-} and SeO_4^{2-}) on plant growth and the content of primary and secondary metabolites in lettuce leaves (*Lactuca sativa* L.) of different age under different lighting (varying percentage of PAR and UV-A in the light stream).

Techniques. The anthocyanin-containing lettuce variety Gurman (*Lactuca sativa* L.) has been selected as a research object. Plants were grown in a greenhouse (Tomsk Region, 2011 and 2014) during the period from May to June with varying proportions of PAR and UV-A in the light stream. Greenhouses were covered with double (F1) or single (F2) polyethylene film. The emission spectrum and relative intensity were measured using a spectrometer AvaSpec-102/256/1024/2048 version 6.2 (Avantes BV, Netherlands).

Seeds were preliminarily treated with 4% solution of sodium selenite or selenate (Sigma, USA) (experiment) or water (control). At the end of the vegeta-

tive stage of 60-day plants, the following parameters were determined: morphological parameters (dry weight and dimensions of sprout structural elements), biochemical parameters, i.e. the content of reducing sugars [14], proteins [15], photosynthetic pigments [16], ascorbic acid [17], anthocyanins and the amount of flavonoids [18]. The content of substances was measured using a spectrophotometer UV-1650 (Shimadzu Corp., Japan), a cell with 10 mm optical path length.

The content of reducing sugars (RS) in plants was evaluated spectrophotometrically [14]. The sample weight of leaves was extracted three times with distilled water at a temperature of 70-80 °C. An aliquot of supernatant was taken from the combined extract and heated in the presence of an alkaline solution of potassium ferricyanide (15 min at 100 °C). A solution of ferrous sulfate mixed with gelatin was added to the cooled mixture. The optical density (OD) of blue-colored solution was measured at $\lambda = 690$ nm. The control solution was a sample that went through all analysis stages, but without RS extracts. In order to express the relative solution density in mass units, a calibration curve was plotted for glucose (initial solution concentration 1000 μg/ml).

The quantification of protein was made according to M.M. Bradford [15]. This method is based on the direct binding of Coomassie G-250 with amino acid residues (arginine, tryptophan, tyrosine, phenylalanine and histidine) in protein. Extracts of leaves were mixed with the Bradford reagent at the room temperature, allowed for at least 2-3 min, and OD was measured at $\lambda = 595$ nm. The control solution was a mixture of the same reagents without the extract. The protein content was determined by a calibration graph for 0.01 to 0.10 mg of the standard protein samples (bovine serum albumin).

Leaves of the 20th layer were analyzed in order to determine the content of chlorophylls a and b (Chla and Chlb) and the amount of carotenoids (Car). Pigments were extracted with 96% ethanol three times. The extract was centrifuged at 10,000 g for 10 min. The optical density of the supernatant was measured using a spectrophotometer at 470, 648.6, 664.2 and 720 nm (values of the last measurement were subtracted from the previous ones to account for possible diffusion). The following formulas by Lichtenthaler were used for calculation of the concentrations of pigments (C_a, C_b, $C_{car.}$) in 96% ethanol (mg/l):

$$C_a = 13.36 \cdot OD_{664.2} - 5.19 \cdot OD_{648.6};\ C_b = 27.43 \cdot OD_{648.6} - 8.12 \cdot OD_{664.2};$$

$$C_{car.} = (1{,}000 \cdot OD_{470} - 2.13 \cdot C_a - 97.64 \cdot C_b)/209.$$

The pigment content per unit leaf area (mg/dm^2) was calculated basing on the data of spectrophotometric analysis given the surface area of cut-outs taken for study.

The content of ascorbic acid (AsA) was determined spectrophotometrically [17]. Fresh leaves were extracted with 50 mM solution of oxalic acid (OA). The extract in equal volumes was mixed with the phosphotungstic reagent (PTR, pH = 1.0), aged for 30 min at 20-25 °C, and centrifuged (7,000 g, 10 min). The optical density of the supernatant (OD_x) was measured at $\lambda = 700$ nm relative to the control solution PTR:OA = 1:1 (v/v). The AsA content (C_x, rM) was calculated according to the formula: $C_x = (OD_x/OD_s)\ C_s$, where C_s is the concentration of the standard solution (56.8 μM L-Ascorbic acid), OD_s − optical density of the standard solution.

The quantity of anthocyanins (Ant) in plant raw material was evaluated as described in [18]. The sample weight was extracted three times with 1% HCl at a temperature 40-45 °C; the combined extract was centrifuged at 10,000 g for 10 min. The optical density of the supernatant was measured spectrophotometrically at $\lambda = 510$ nm (control solution 1% HCl). The amount of anthocyanin (%) in terms of cyanidin-3,5-diglycoside in absolutely dry raw materials ($X_{ant.}$) was calculated according to the formula: $X_{ant.} = OD \cdot 250 \cdot 100 \cdot 453^{-1} \cdot m^{-1} \cdot (100 - W)^{-1}$,

where OD is the optical density of the experimental solution; 453 is specific absorbtion of cyanidin-3,5-diglycoside in 1% HCl; m is raw weight; W is loss in weight after drying, %.

Flavonoids (Fl) were determined spectrophotometrically [18]. The sample of plant raw material was extracted three times with 70% ethyl alcohol on a boiling water bath for 60 min, the extracts were combined. An aliquot of the extract was aged in the presence of aluminium chloride and acetic acid, and after 40 min, optical density was determined at λ = 415 nm. The control solution did not contain aluminum chloride and was prepared for each sample separately. Scheme for OD measurement of control rutin solution was the same. The total content (%) was re-calculated per rutin and absolutely dry weight ($X_{flav.}$):

$$X_{flav.} = OD_x \cdot K_x \cdot m_x^{-1} \cdot m_p \cdot OD_p^{-1} \cdot K_p^{-1} \cdot 100 \cdot (100 - W)^{-1} \cdot 100,$$

where OD_x is the optical density of the experimental solution; OD_p is optical density of the rutin solution; m_x raw weight of biomaterial, g; m_p is weight of rutin, g; K_x is dilution factor of the experimental solution (1250); K_p is dilution factor of the rutin solution (2500); W is loss in weight after drying, %.

The physiological condition of leaves in different layers was assessed according to changes in growth processes. The leaf length and width on plants with the same number of layers as in test plants were measured 5 days prior to estimation of the main indicators with subsequent plotting of growth curves. The leaf area was measured using photographs (Moticam 3.0 software, Motic, Netherlands).

The results were statistically processed using the Student and Fisher criteria (Microsoft Excel 2007standard software). The figures represent the arithmetical average (M) for the growth (n = 50) and biochemical (n = 5) parameters with double-sided confidence intervals (M±1.96 SEM). The growth parameters were analyzed independently in the same organ or layer of leaves, the contents of pigments in a mixed group. Differences between values marked by different letters are statistically significant at $p < 0.05$. Changes of the studied morphological and physiological indicators of plants have similar dynamics, so the article contains data for one year 2014.

Results. Plants are characterized by long-lasting growth throughout the whole life. The growth of sprouts is ensured by the formation of new metamers, between which donor-acceptor relations remain. The sprout has actively growing (young) leaves, already grown leaves, actively functioning (adult) leaves and aging leaves with elements of chlorosis. Regulation of the size and shape of the sprout is one of the adaptation mechanisms of plants to environmental conditions. The response of the whole plant to an external factor seems to be more complex than the response of a separate organ. Therefore, the authors have conducted studies of a multilayered sprout, rather than a single metamer (layer) of the plant.

Changes of PAR and UV-A during the experiment are illustrated (Fig. 1, A). The optical properties of F2 were characterized by a greater light-permitting ability than F1: in the range λ = 360-390 nm (UV-A), the differences accounted for 40-50%, λ = 400-500 nm (blue light) for 29-35%, λ = 500-600 nm (green light) and λ = 600-700 nm (red light) for 30%. At the initial stages of ontogenesis (on day 27), donor-acceptor relations between the consistently forming structural elements of the sprout, leaves of different layers, were transforming, which was reflected in a change of their dry weight (see Fig. 1, B). Control plants cultivated without selenium have demonstrated earlier completion of growth of the 1st layer leaves under F1, which has caused longer growth of subsequent leaves of the 2nd and 3rd layers (by 4.2 and 3.8 times, $p < 0.05$). Growth inhibition of these layers under F2 has led to the accumula-

tion of dry mass in the 4th and 5th layers (by 4.6 and 3.8 times relative to the 1st layer). This kind of redistribution of the growth processes in plants under F2 resulted in a greater increase in the sprout size, which was accompanied by the development of new metamers while maintaining the total surface area of leaves in control plants compared with those under F1 (see Fig. 1, B). As a result, 60-day plants had 21 layers under F1 and 24 layers under F2.

Pre-seeding treatment of seeds with SeO_3^2 has slowed down the accumulation of dry matter by leaves of the 1st-2nd layers (20% to the control), which contributed to the growth of the leaves of the following layers, while SeO_4^2 has led to a 3.4 times more longer growth of the first leaf and inhibited growth of subsequent leaves (see Fig. 1B, F1). An increase in the percentage of PAR and UV-A in the light stream has changed the sprout response to Se. SeO_3^2 hindered the growth of leaves of the 1st and 4th-5th layers (by 36, 40 and 17%, respectively, $p < 0.05$), whereas SeO_4^2 accelerated the growth of leaves of the 1st-2nd layers by 39 and 88%, respectively, compared to the control under F2 ($p < 0.05$).

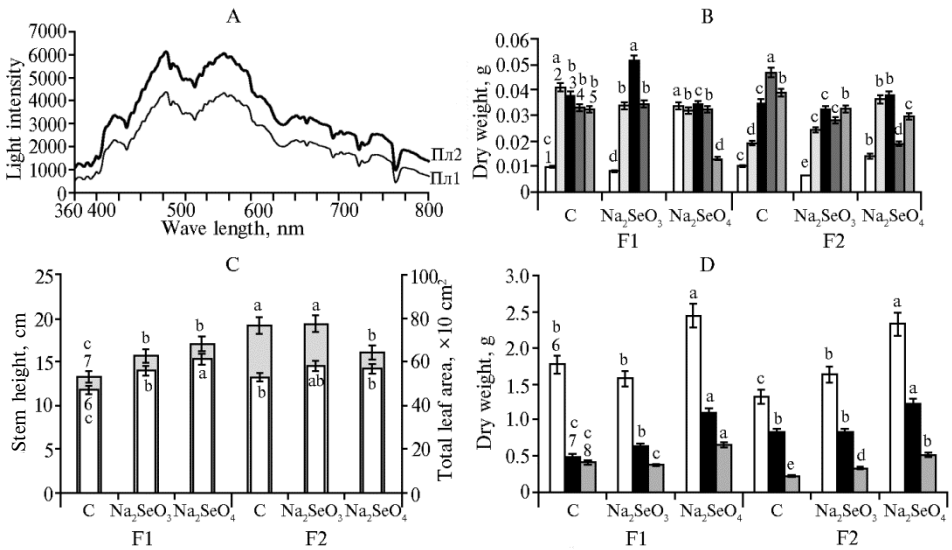

Fig. 1. Light spectra in greenhouses under films F1 and F2 (A) **and the growth parameters of leaves of the 1st-5th layers** (B) **and organs** (C, D) **in 27-day** (B) **and 60-day** (C, D) **lettuce (*Lactuca sativa* L.) plants cv. Gurman depending on light intensity and pre-seeding treatment with Se:** 1-5 — leaves of the 1st-5th layers, 6 — leaves of all layers, 7 — stem, 8 — root (Tomsk Province, average for the year 2014). Vertical bars indicate ±1.96 SEM ($n = 50$). Growth parameters were analyzed independently for the same layer of leaves (B) or organ (C, D). For each parameter, differences in values marked with different letters are statistically significant at $p < 0.05$.

With the completion of the vegetative development stage, SeO_4^2 increased the dry mass of leaves, stems, and roots in experimental 60-day lettuce plants relative to the control (see Fig. 1, D). Ions of SeO_4^2 had an advantage in the regulation of sprout development compared to SeO_3^2. Leaves form more layers under the influence of SeO_4^2 rather than SeO_3^2, 24 and 21 for F1, 24 and 28 for F2, respectively. Treatment with SeO_4^2 provided a stimulating effect (+15%) similar to light under F1 and an additive effect (+33%) after an increase in the percentage of PAR and UV-A under F2. Other authors have shown greater efficiency of SeO_4^2 (2-4 μM) compared to SeO_3^2 (6-10 μM) in the regulation of the leaf area in *Cucumis sativus* L. [19]. The age dependence of growth processes on the concentration of SeO_4^2 has been demonstrated: addition of low concentrations did not affect the raw or dry weight of younger plants *L. sativa*, but signifi-

cantly stimulated growth in aging plants [20].

Differences in growth processes in plants in response to light of different quality could be due to metabolic changes. Sugars as primary exchange products are necessary for growth and differentiation. The sugar content in leaves has been changing depending on their functional status (age) and the intensity of photosynthesis. For control plants *L. sativa* under F1, the authors have identified a higher content of reducing sugars (RS) in grown leaves, where an active synthesis of these compounds took place. Low accumulation of RS has been noted in the actively growing (17th-21th layers) and aging (4th-7th layers) leaves (Fig. 2, A) since the former acted only as acceptors of sugars, and the latter were dying. An increase in the proportion of PAR and UV-A in the light stream (F2) resulted in an increase in RS production by 30%, 45% and by 2.3 times in aging, adult (8th-16th layer) and young leaves in control plants compared with those under F1, which could indicate their different physiological status associated with the activation of photosynthesis or increased transport of sugars. A change in hormonal balance is also possible since light-dependent integration of signaling pathways for sugar and hormones through PIF (phytochrome interacting factor) and DELLA (transcriptional repressors of gibberellin signaling) proteins has been demonstrated [21].

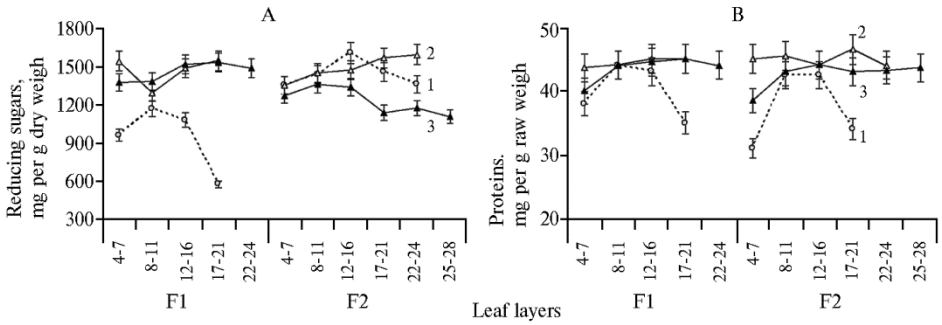

Fig. 2. Reducing sugars (RS, A) **and water-soluble proteins** (B) **in leaves of different layers in 60-day lettuce (*Lactuca sativa* L.) plants cv. Gurman depending on light intensity in greenhouses under films F1 and F2 and pre-seeding treatment with Se:** 1 — control, 2 — sodium selenite, 3 — sodium selenate (Tomsk Province, average for the year 2014). Vertical bars indicate ±1.96 SEM (n = 5).

According to the research results, the amount of RS increased in young lettuce leaves and remained high in aging leaves under F1 under the influence of SeO_3^2 and SeO_4^2 ions. The increase in the RS content in old lettuce leaves (4th-7th layers) could indicate a withdrawal of their aging effects and activation of photosynthetic reactions. Such effect of Se could be connected to the restoration or maintenance of the structure of cell membranes and integrity of cells by reducing the amount of O_2 and H_2O_2 after adding of this element [1]. The role of SeO_4^2 in the acceleration of photosynthesis is confirmed by the results by M. Djanaguiraman et al. [22] obtained for sorghum plants. The increased content of soluble sugars and starch has been also described for potato leaves after treatment with Se [4]. Under F2, the effectiveness of SeO_3^2 increased only in young lettuce leaves, and the effectiveness of SeO_4^2 decreased in adult and young leaves. The observed reactions of lettuce plants showed the influence of light on the accumulation of Se and the influence of the element on the duration of synthetic processes in leaves of different layers. It is known that the travel rate of SeO_3^2 in plants is lower than that of SeO_4^2, and the impact of red and blue light in addition to white light increases the amount of endogenous Se [20, 10].

The authors hereof have shown that in plants *L. sativa* under F1, the highest content of soluble proteins (see Fig. 2, B) was in grown leaves (8th-16th

layers). With an increasing proportion of PAR and UV-A in light (F2), the amount of proteins decreased in aging leaves of control plants, which was accompanied, however, by an increase in carbohydrate metabolism (see Fig. 2, A). Se increased the protein content in young (17th-28th layers) and aging (4th-7th layers) leaves regardless of light spectral composition. SeO_3^2 supported the content of proteins in aging leaves better than SeO_4^2, since it is known that selenite is more effective than selenate as an inductor of the activity of antioxidant enzyme selenium-dependent glutathione peroxidase (GSH-Px) [23].

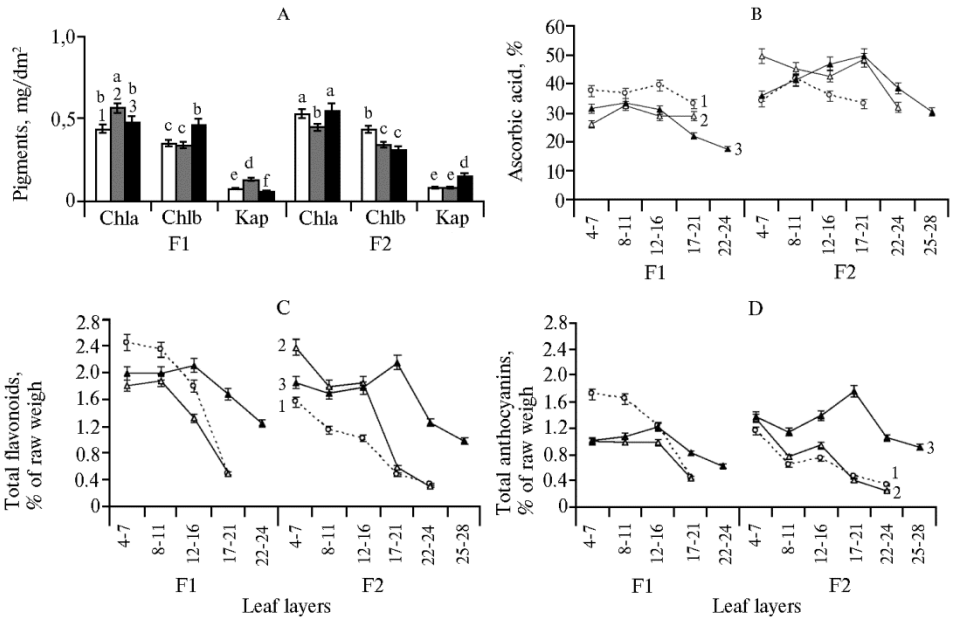

Fig. 3. Photosynthetic pigments in leaves of the 20th layer (A), **distribution of ascorbic acid** (B), **flavonoids** (C) **and anthocyanins** (D) **in leaves of different layers in 60-day lettuce (*Lactuca sativa* L.) plants cv. Gurman depending on the light intensity in greenhouses under films F1 and F2 and preseeding treatment with Se:** 1 — control, 2 — sodium selenite, 3 — sodium selenate, Chla and Chlb — chlorophylls a and b, Car — carotenoids (Tomsk Provice, average for the year 2014). Vertical bars indicate ±1.96 SEM (n = 5). The pigment content was analyzed in a mixed group. For pigments, differences in values marked with different letters are statistically significant at $p < 0.05$.

Accumulation of photosynthetic pigments in leaves of the 20th layer (Fig. 3, A) varied depending on the light intensity and spectral composition. The growing proportion of PAR and UV-A in the light (F2) caused an increase in Chla and Chlb compared to control plants under F1. The total content of all photosynthetic pigments in different variants under F1 and F2 was higher than the control for F1. However, the total number of chlorophylls influenced by SeO_3^2 for F2 stayed within the F1 control and below the F2 control. The individual pigment composition of leaves also depended on the form of selenium. SeO_3^2 under F1 increased the content of carotenoids by 60% ($p < 0.05$) that act as antioxidants and protect Chla from photochemical oxidation [24]. This contributed to the accumulation of Chla under F1, whereas SeO_4^2 decreased the carotenoid content and increased the amount of the oxidized Chlb form. The expression of the antioxidant effect of SeO_4^2 similar to SeO_3^2 under F1 occurred with an increasing proportion of PAR and UV-A radiation in the light stream (F2). Perhaps the latter conditions increased the absorption of selenium or its restoration.

Ascorbic acid is an important antioxidant in plant tissues [25]. Most of its content is located in grown leaves of the 12th-16th layers (F1) and grown leaves of the 8th-11th layers (F2). Both forms of selenium hindered the synthesis

of AsA under F1, whereas with an increasing proportion of PAR and UV-A in the light stream (F2), SeO_3^2 increased the content of AsA in aging and adult leaves, and SeO_4^2 in adult and young leaves (see Fig. 3, B). These results are consistent with the data on the increase in AsA in leaves and chloroplasts during acclimatization to high-intensity light [26]. AsA deficit in *vtc* mutants of *Arabidopsis* reduces the zeaxanthin-dependent non-photochemical quenching supported by violaxanthin de-epoxidase and determines sensitivity to photooxidation. Processing with exogenous AsA reduces the phytotoxic effect of high concentrations of Se that is manifested in relation to the membrane, chlorophyll and PSII functions in plants *Oryza sativa* L. through an increase in the activity of antioxidant and metal-tolerant mechanisms [27]. In the first mechanism, the effect is due to the action of enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), as well as non-enzyme antioxidants, i.e. AsA, glutathione, and proline. The second mechanism is implemented through metallothioneins, thiols, and glutathione-S-transferase (GST). An increase in the content of these molecules reduces the toxic effect of Se through its conjugating and/or removal of reactive oxygen species generated due to selenium stress. Low Se concentrations (1 mg/kg) increase the activity of SOD, CAT, APX, and GR [27].

Flavonoids are essential among the secondary metabolites. The authors have established the age dependency of Fl accumulation in leaves of *L. sativa* plants (see Fig. 3). In adult and aging leaves, flavonoid accumulation was 4 and 6 times higher than in young leaves (17th-21th layers). This meant the strengthening of the synthesis of secondary metabolites with the completion of the active leaf growth. The obtained data are consistent with reports on photoinhibition of flavonoid biosynthesis at an early stage of development of the *Gossypium hirsutum* fiber *in vitro*, but stimulating at later stages [28].

Pre-seeding treatment with SeO_4^2 increased 4-fold the total amount of flavonoids in leaves compared to control leaves and plants treated with SeO_3^2 in variants with F1. With an increasing proportion of PAR and UV-A (F2), the stimulating effect of both Se forms on the accumulation of flavonoids in plants was observed. Other authors also describe Se as a photoprotector from harmful UV-B radiation [27], which manifests itself in *Triticum aestivum* L. primarily as increasing amounts of antioxidants and a decrease in the membrane peroxidation of lipids (MPL) in aboveground parts of plants. Se provides an ambiguous effect on MPL in roots: it inhibited peroxidation at low concentrations and intensified it at high concentrations [19, 29].

Anthocyanins (Ant) as flavonoids have been accumulated in grown leaves of the 4th-11th layers of control plants under F1 (see Fig. 3). A simultaneous increase in the proportion of the visible spectrum and UV-A (F1) has reduced the amount of Ant in the control. Under F1, the treatment with SeO_4^2 has increased its content in young leaves of the 17th-24th layers and reduced in adult leaves compared with the controls. Treatment with SeO_4^2 under F2 has increased the content of Ant in leaves of the most layers (see Fig. 3). At the same time, a decrease in the accumulation of RS has been observed (see Fig. 2, A). The latter fact can be explained by the role of substrate sugars in the synthesis of Ant. However, partial coherence in changes of the content of RS (see Fig. 2, A), Fl (see Fig. 3A) and Ant (see Fig. 3, B) in other circumstances was probably caused by a signal function of sugars regulating the expression of genes that control the biosynthesis of flavonoids and Ant. Other authors have shown that sugars activate the gene *PAP1* (*Production of Anthocyanin Pigmentation 1*) through a sugar-specific signaling pathway [30]. At that, not RS, but sucrose increased the synthesis of *PAP1* mRNA and the expression of genes encoding enzymes of Ant biosynthesis, the *DFR*, *LDOX*,

and *UF3GT* (*Dihydroflavonol-4-Reductase*, *Leucoanthocyanidin Dioxygenase*, *UDP-Glucose: Flavonoid 3-O-Glucosyltransferase*) determining Ant accumulation.

Based on the obtained results, the authors have suggested that differences in the metabolism of *L. sativa* plants depending on lighting are caused by the specific functioning of regulatory photoreceptors. An increase in the proportion of PAR and UV-A in light (F2) has increased the contents of photosynthetic pigments and carbohydrate in the leaves of control plants, which accelerated the development of sprouts. This is consistent with the data on elimination of UV-A-induced negative effects on photosynthesis, PSII activity and the contents of photosynthetic pigments, with preliminary exposure to the red light that is associated with the phytochrome control of these reactions [31]. Specific light-dependent response of plants to Se can be associated with the unequal accumulation of different forms of its ions, because additional exposure to the red and blue light in addition to white increases the amount of endogenous Se in plants [10]. It is known that the growth-enhancing effect of SeO_3^2 exists in a more narrow range of concentrations than in the case of SeO_4^2 (respectively 6, and 6-20 μm) [19]. Another explanation for the different direction and rate of growth processes can be the fact that Se as a pro- or antioxidant changed the accumulation of Fl of various nature. Flavonoids with o-hydroxyls in the nucleus acted as auxin synergists stimulating the growth of plants as a result of inhibition of IAA-oxidase, while Fl with p-hydroxyls acted as cofactors of IAA-oxidase demonstrating the properties of IAA antagonists and, consequently, being growth inhibitors [32]. Se could affect the content of other phytohormones [33], and hormones, in turn, could alter the Se-dependent growth of plants [34].

The differential response of lettuce leaves in different layers depended on age (primarily on the oxidative status, which was determined by the content of metabolic or stress ROS). Other authors [20] have demonstrated the ability of SeO_4^2 to counteract the aging-induced oxidative stress in *L. sativa*. In young and aging plants, the antioxidant effect of Se is associated with the increased activity of glutathione peroxidase (GSH-Px). In aging plants, an increase in the amount of Se enhances the antioxidant ability, preventing a decrease in the concentration of α-tocopherol and increasing SOD activity.

Thus, it has been established that the pre-seeding treatment of *Lactuca sativa* seeds with Se in two ionic forms regulates the intensity of growth and metabolic processes in plants, changing the content of primary and secondary metabolites. The pre-seeding treatment of seeds with Se provided a stimulating effect on the formation of new metamers of *L. sativa* sprouts and seed germination (SeO_4^2). At the same time, the regulation of the light stream spectrum has changed the efficiency of Se. An increased proportion of PAR and UV-A has stimulated growth processes (stretching and thickening of the sprout, formation of new metamers) through the activation of carbohydrate metabolism. The age dependence of the morphological and physiological parameters of leaves in *L. sativa* plants on the forms of selenium and light spectrum has been demonstrated. Under F1, at a lower intensity of UV-A and PAR, after the treatment with Se, the content of reduced sugars and proteins increased in actively growing leaves and remained the same in old ones. SeO_4^2 increases the amount of flavonoids regardless of the light spectrum, whereas SeO_3^2 has the same upon an increase in the proportion of PAR and UV-A. A higher proportion of PAR and UV-A can probably result in Se metabolism acceleration of. Maybe there was also a protective effect of high PAR with increasing UV-A, which resulted in the enhancement of carbohydrate metabolism and an increase in the content of ascorbic acid. Our findings extend the understanding of plant adaptive response to light of varying quality by providing better understanding of Se-dependent mechanisms

defining resistance to increased proportions of PAR and UV-A in light. The obtained data can also be used for diagnosis of the physiological condition of leaves in different layers with and without selenium. Pre-seeding treatment with Se combined with a changing light spectrum increases the nutritional value of lettuce due to the accumulation of primary and secondary metabolites.

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