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BIOSYNTHESIS OF RUBBER AND INULIN DEPENDING ON THE SPECTRAL COMPOSITION OF LIGHT AND ACTIVITY OF THE PHOTOSYNTHETIC APPARATUS DURING AEROPONIC CULTIVATION OF *Taraxacum kok-saghyz* E. Rodin

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Abstract

Due to the intensive development of industry and new technologies, the demand for natural rubber is increasing. The synthetic rubber cannot replace this biopolymer due to its unique consumer and operational characteristics. Along with the traditional source of natural rubber production from the latex of Brazilian Hevea *Hevea brasiliensis* (Willd. ex A.Juss.) Müll. Arg., work is underway to obtain it from kok-saghyz plants *Taraxacum kok-saghyz* E. Rodin which can be grown both in natural and controlled conditions. The determination of the most favorable light conditions, taking into account the physiological state of plants, is important to obtain a high yield of target products. In this study, we have shown for the first time an increase in the rate of rubber biosynthesis when irradiating kok-saghyz plants with light with a greater proportion of blue spectrum. The paper also describes the changes in light and dark reactions of the photosynthetic apparatus, and in sucrose and glucose accumulation in plants when changing the light regime for several hours. The aim of the work was to study the influence of light conditions on the physiological and biochemical processes and biosynthesis of rubber and inulin in kok-saghyz plants grown under controlled phytotron conditions. Kok-saghyz seeds (a collection form 391 from the VIR collection of the Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg) were germinated under sterile conditions. From days 19-20, when 3-4 true leaves appeared, the plants were grown in the aeroponic phytotron with full-spectrum light-emitting diodes (LED) of photosynthetic active radiation PAR_{400-700 nm} of $400 \pm 28 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In the first chamber of the phytotron, there was a $255.6 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ exposure for PAR_{400-700 nm} blue spectrum (BS, $\lambda_{\text{max}} = 460 \text{ nm}$) and $75.6 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for PAR_{600-700 nm} red spectrum (RS, $\lambda_{\text{max}} = 660 \text{ nm}$), with the RS/BS ratio of 0.30. In the second chamber, the RS irradiation intensity

(PAR_{600-700 nm}) was 259.6 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the BS irradiation intensity (PAR_{400-500 nm}) was 71.8 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, with the RS/BS ratio of 3.6. The revealed parameters of growth, photosynthetic activity, and accumulation of glucose and sucrose in leaves and rubber and inulin in roots under various spectral modes during long-term growth and with changing the irradiation mode draw us to the following conclusions. When growing plants for 28 days in phytotron chambers with an increased proportion of RS in the spectrum, the content of rubber increased 3-fold, of inulin 4.1-fold compared to the initial values. With an increase in the BS proportion in PAR, the levels of rubber and inulin rose 5.4 times and 4.6 times, respectively. Ultimately, when irradiated with light with a higher proportion of BL, plants accumulated 1.75 times more rubber. The change in the irradiation spectrum from BS to RS led to a short-term increase in the concentration of glucose and sucrose in the leaves compared to the initial values. This dependence persisted for 2 hours, after which the sucrose content did not change, but there was a decrease in glucose content. When the irradiation mode changed from BS to RS, the activity of the photosynthetic apparatus decreased, i.e., the rate of photosynthesis from 26.7 to 15.2 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at light saturation, the rate of dark respiration from 2.80 to 2.38 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and the quantum yield of photosynthesis from 0.066 to 0.055. Switching from RS to BS led to opposite results. It follows from the obtained data that the change in the concentration of soluble carbohydrates in plants is associated with a change in the spectral composition of irradiation and, as a consequence, with a change in the activity of the photosynthetic apparatus. When the irradiation changed from RS to BS, there was an increase in the rate of photosynthesis and activity of photosystem II, but a decrease in the accumulation of glucose and sucrose during the first 2 hours with a return to the initial values after 3 hours.

Keywords: *Taraxacum kok-saghyz*, kok-saghyz, growth, rate of photosynthesis, dark transpiration, quantum yield of photosynthesis, rubber, inulin, LED light sources, phytotron, aeroponics

Secondary plant metabolites perform many important functions and are widely used by humans. Well-known compounds are alkaloids, polyphenols, including flavonoids and terpenoids, inulin, and rubber. Many of them are irreplaceable (artificial synthesis is impossible) and are used for medical, nutraceutical purposes, as well as in industry [1, 2]. Studying the influence of various factors on the synthesis, accumulation and localization of substances of secondary metabolism remains one of the pressing problems of modern plant physiology [3-5].

Currently, all over the world, due to the intensive development of industry and new technologies, the demand for natural rubber (NR, the 1-4-cis-polyisoprene) is increasing. NR is 91-96% polyisoprene, contains proteins, amino acids and fatty acids and cannot always be replaced with synthetic analogues. NR has unique consumer and operational characteristics (impact resistance, wear resistance, effective heat dissipation) [6-9], is highly elastic and, even under the influence of low forces, has a reversible tensile deformation of up to 1000%. The elasticity of rubber is maintained over a wide temperature range; this is its characteristic property, therefore, for example, in the automotive industry, tires are used in the production of which the composition of the materials includes up to 20% of natural rubber, while in aircraft tires its share is up to 100% [10]. NR is used in tire casings where high strength is required, and synthetic rubbers are used in tread materials to provide tire traction [11].

The main economically significant source of industrial production of NR was and remains latex from *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. [12]. However, the increasing demand for the product is becoming an incentive to search for alternative raw materials for the production of natural rubber [13, 14] and to study the biochemical and molecular genetic aspects of its biosynthesis [15, 16]. One of the promising ones may be the rubber plant kok-saghyz (*Taraxacum kok-saghyz* E. Rodin), which was widely grown in the USSR in the 1930-1950s [17]. In addition to rubber, inulin can be obtained from kok-saghyz, which makes up up to 35% of the dry weight of the roots [18]. In field conditions, kok-saghyz is cultivated for 2 years. Under phytotron conditions, the period of collecting root biomass begins after 2 months of growing plants and lasts for quite a long time, during which part of the root system is periodically cut off. After each such procedure, the plants regenerate the root system to its previous size within 28-30 days,

maintaining the functions of active biosynthesis of rubber and inulin. Therefore, along with growing plants in open ground conditions, it seems possible to obtain a high yield of the target product year-round, under controlled conditions using artificial irradiation sources [19].

In kok-sagyz plants, rubber is synthesized in specialized structures, the laticifers. Part of the NR accumulates in the milky sap, mainly in the roots. By the end of the growing season, the amount of rubber in the roots increases significantly. On average, from 7 to 15% of rubber accumulates in plant roots, and in some collection samples up to 25% of the dry weight of the roots [20, 21]. In this case, the inulin content reaches 30-35% of the dry weight of the root.

To date, the light regime (intensity, spectral composition, duration of the photoperiod) for growing kok-saghyz remains unstudied, and the determination of the most favorable light conditions, taking into account the physiological state of plants, is considered as an important element in increasing the rate of biosynthesis of rubber and inulin.

The results presented in this work provide initial information about the influence of the spectral composition of irradiation on the accumulation of rubber and inulin, as well as on the activity of the photosynthetic apparatus during long-term cultivation of kok-saghyz when irradiated with light with different spectral composition. For the first time, it is shown that the rate of rubber biosynthesis increases under irradiation with a greater proportion of the blue part of the spectrum. We also revealed changes which occurred in the speed of light and dark reactions of the photosynthetic apparatus and the concentrations of sucrose and glucose when modulating the light regime during several hours.

The purpose of the work is to study the influence of light conditions on physiological and biochemical processes and the biosynthesis of rubber and inulin in kok-saghyz plants grown under controlled phytotron conditions.

Materials and methods. The studies were carried out in phytotrons with controlled light irradiation, temperature, humidity, mineral nutrition, and gas composition. Kok-sagyz plants (k-391, VIR collection, the Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg), grown from seeds under sterile conditions, on days 19-20 (3-4 true leaves) were planted in aeroponic phytotrons, in two chambers with full spectral LED irradiation in the region of photosynthetically active radiation (PAR_{400-700 nm}) $400 \pm 28 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In the first chamber, irradiation in the PAR_{400-500 nm} of the blue spectrum ($\lambda_{\text{max}} = 460 \text{ nm}$, LED BS) was $255.6 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, in the PAR_{600-700 nm} of the red spectrum ($\lambda_{\text{max}} = 660 \text{ nm}$, LED RS) $75.6 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In the second chamber, the RS intensity of PAR_{600-700 nm} was $259.6 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the BS intensity of PAR_{400-500 nm} was $71.8 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Air temperature, humidity, and CO₂ concentration were monitored using a wireless sensor E+E EE244 (E+E Elektronik, Austria) integrated into the control system in the phytotron. The CO₂ concentration in both chambers was $418 \pm 15 \mu\text{mol/mol}$, air humidity was 60-80%, temperature was $22 \pm 1 \text{ }^\circ\text{C}$. Spectral regimes of irradiation were determined using an ASENSEtek PG100N spectrometer (UPRtek Corp., Taiwan). Measurements were carried out in 0, 1, 2, and 3 hours under a stationary light regime with predominant blue or red light, as well as after changing one predominant light to another.

The rate of photosynthesis was studied using an infrared gas analyzer CPro+ (ADC BioScientific Ltd., UK) to assess CO₂ exchange directly in chambers under given light conditions for growing plants. The dependence of the rate of CO₂ exchange on light intensity was determined for 0 to $1600 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the air CO₂ concentration $418 \pm 15 \mu\text{mol/mol}$ upon successively increasing the light intensity. To describe the light curve of photosynthesis, we

used the equation of an irregular hyperbola [22].

The activity of photosystem II (PSII) was determined by the variable fluorescence method using a compact minifluorimeter JUNIOR-PAM (Heinz Walz GmbH, Germany). Leaves of kok-saghyz plants were left in the dark for 15 min, then illuminated with flashes of light [23, 24]. The functional state of PSII, the maximum quantum yield of PSII, the effective quantum yield of PSII at the irradiation intensity during the fluorescence measurement, non-photochemical fluorescence quenching which estimates the part of the energy that is used by the plant for non-photochemical reactions, the relative rate of electron transport as an indirect indicator of the rate of photosynthesis were determined.

For quantitative analysis of glucose and sucrose, the middle parts (0.5 g) of fully developed kok-saghyz leaves (without the central vein) were homogenized manually in a mortar with 4.5 ml of water, and then centrifuged for 20 min at 6000 rpm (FC5718, OHAUS Corporation, USA). The resulting extract was used to quantify water-soluble low molecular weight sugars. It was previously shown that there are no low molecular weight carbohydrates in the sediment.

To quantify the glucose concentration, the standard Glucose-Novo kit (Vector-Best, Russia) for the analysis of liquid substrates was used. After adding reagents to the extract, it was incubated for 20 minutes at 50 °C. The optical density (OD) of the solution was measured at $\lambda = 508$ nm and $\lambda = 343$ nm (a spectrophotometer UV2600, Shimadzu, Japan) and the glucose concentration was calculated. The GOPOD (glucose oxidase peroxidase) method was used based on measurement of the color resulting from the reaction of the chromogen with hydrogen peroxide, which, in turn, serves as a product of glucose oxidation with atmospheric oxygen in the presence of gluco-peroxidase [25].

When determining the amount of sucrose, the enzyme invertase (Available Carbohydrates Assay Kit, Megazyme cat. no. K-AVCHO, Megazyme, Ltd., Ireland) was added to the extract to hydrolyze sucrose into glucose and fructose. Then the sample was incubated for 30 min at 30 °C and pH 6.5. After the hydrolysis reaction was completed, free glucose and glucose formed during the enzymatic hydrolysis of sucrose were specifically stained. To determine the sucrose concentration, the difference between the obtained concentration and the previously determined free glucose concentration was calculated [26].

The rubber content was assessed gravimetrically. The rubber was isolated as described by D.A. Ramirez-Cadavid et al. [27]. Freshly collected roots were washed with water, dried for 24 hours at 65 °C, ground in a mortar, and the powder was sifted on a 1×1 mm sieve. A sample of root powder (300 mg) was used for extraction. Extraction with chloroform (40-45 ml, grade CP, OOO TD HIMMED, Russia) was carried out (a Soxhlet extractor, OOO MLS Klin, Russia) for 6 hours. The resulting extract was concentrated by evaporation at 75 °C to a residual volume of 3-5 ml. Rubber from an aliquot of the extract was precipitated with a triple volume of ethanol at 4 °C for 15-16 hours in a tared microtube. The precipitate was separated by centrifugation for 20 min at 14,500 rpm (Eppendorf 5424, Eppendorf, Germany). Associated substances were removed from the rubber sediment by sequentially washing the sediment with distilled water and then with acetonitrile (Sigma, USA) followed by centrifugation. Residues of water and acetonitrile were removed by washing the precipitate three times with ethyl alcohol, followed by centrifugation. The purified rubber was dried by blowing with argon. Based on the dry root biomass, the percentage of rubber from the mass of the sediment was calculated.

To determine the amount of inulin, the roots were washed with water, dried for 24 hours at 65 °C, ground in a mortar, and the powder was sifted on a 1×1 mm sieve. A water bath was used for aqueous extraction of inulin (85-90 °C

for 60 minutes) from a 200 mg sample of root powder. The suspension was centrifuged at 14,500 rpm for 5 min or filtered through a glass fiber membrane with a pore size of 1 μm . Acid hydrolysis of inulin was carried out with 10% HCl in a 1:1 ratio (sample:acid) for 60 minutes in a boiling (100 °C) water bath. The Nelson-Somogyi method was used (GOST R 54905-2012. M., 2013) to determine reducing sugars before and after hydrolysis (a UV-1202 spectrophotometer, Shimadzu, Japan) [28].

Experiments were performed in 4-5-fold analytical and 3-fold biological replicates. The general pattern did not change depending on the experimental variants; therefore, the results are presented based on data from one biological replicate. Statistical processing of the results was carried out using the Microsoft Excel software package. The figures and table show the arithmetic mean values (M) with standard error ($\pm\text{SEM}$). The significance of differences was determined by Student's t -test at $P = 0.95$.

Results. Light is a key factor in the growth and development of plants whose photoreceptors evaluate the quality (spectral composition) and quantity (intensity) of light and adapt to these parameters. Light signals, sensed primarily by phytochromes and cryptochromes, regulate plant growth, metabolic, and morphogenetic responses [29].

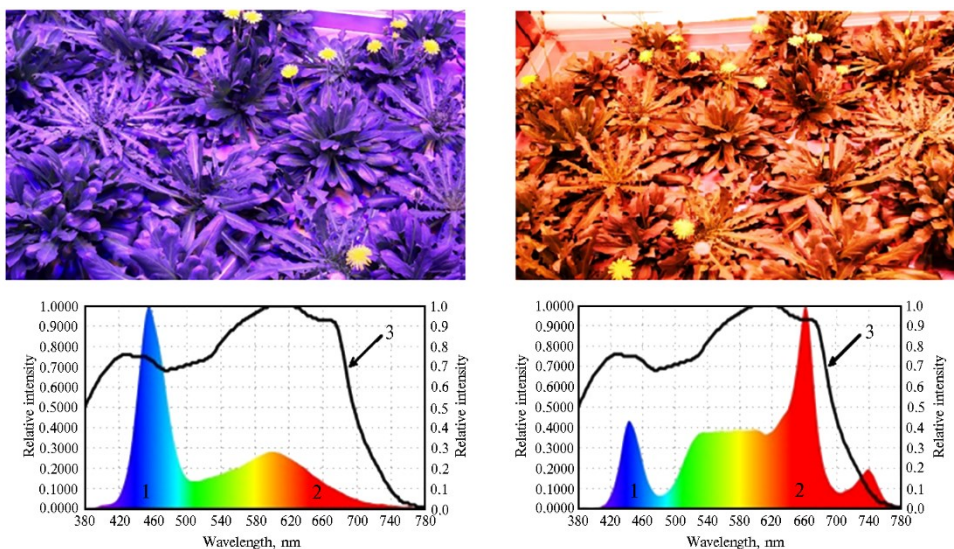


Fig. 1. Spectral characteristics of LED irradiators during long-term aeroponics cultivation of kok-saghyz plants (*Taraxacum kok-saghyz* E. Rodin) in the chambers of an aeroponic phytotron: 1 — PAR₄₀₀₋₅₀₀ nm of the blue spectrum, 2 — PAR₆₀₀₋₇₀₀ nm of the red spectrum, 3 — curve dependence of the intensity of photosynthesis on the wavelength of incident light (action spectrum of photosynthesis according to K.J. McCree). The radiation intensity was measured at the landing field level.

An increase in the proportion of blue light (Fig. 1, on the left) or red light (see Fig. 1, on the right) when growing kok-saghyz plants in the chambers of the aeroponic phytotron led to a change in the RS/BS ratio in the irradiation spectrum. The RS/BS ratio in the first cultivation chamber was 0.30, in the second chamber 3.60. There were practically no differences in the rate of photosynthesis per unit leaf area, 9.4 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the initial RS (Fig. 2, A, 0 RS) and 10.9 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the initial BS (see Fig. 2, B, 0 BS), respectively.

Changing the light regime from a predominantly red spectrum (RS) to a predominantly blue spectrum (BS) in 1 hour after the switch led to a decrease in the rate of photosynthesis, and in 2 hours and onwards to its increase compared to the initial values (see Fig. 2, A). With the opposite change in the light regime

(from BS to RS), the changes differed. A slight increase in the rate of photosynthesis was followed by a noticeable decrease, but in 3 and 4 hours, the rate of photosynthesis increased, although not as notable as when the spectral mode changed from RS to BS (see Fig. 2, B).

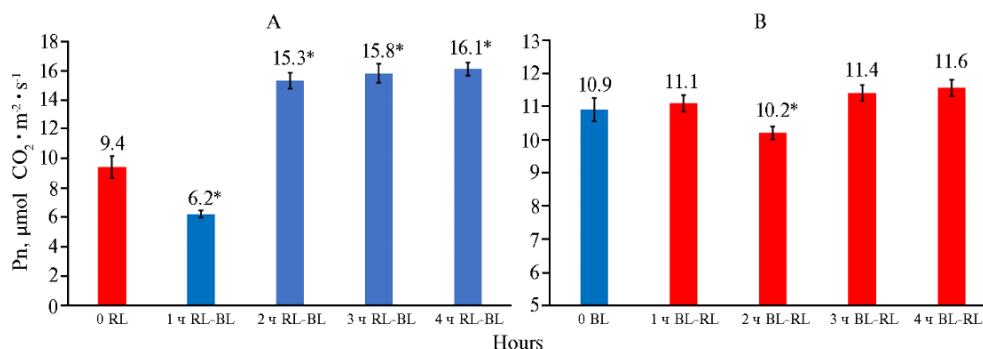


Fig. 2. The rate of photosynthesis in the leaves of kok-saghyss plants (*Taraxacum kok-saghyss* E. Rodin) during long-term cultivation by aeroponics in the chambers of an aeroponic phytotron at a light intensity of $400 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a predominance of red light (RS) (A) or blue light (BS) (B) in the irradiation spectrum, as well as when the irradiation spectrum changes from RS to BS (A) and from BS to RS (B) ($M \pm \text{SEM}$, $n = 5$).

* Differences from RS (A) and BS (B) are statistically significant at $P = 0.95$.

To clarify in more detail the nature of the influence of a change in the spectral regime on the activity of the photosynthetic apparatus, light curves of the rate of plant photosynthesis were measured.

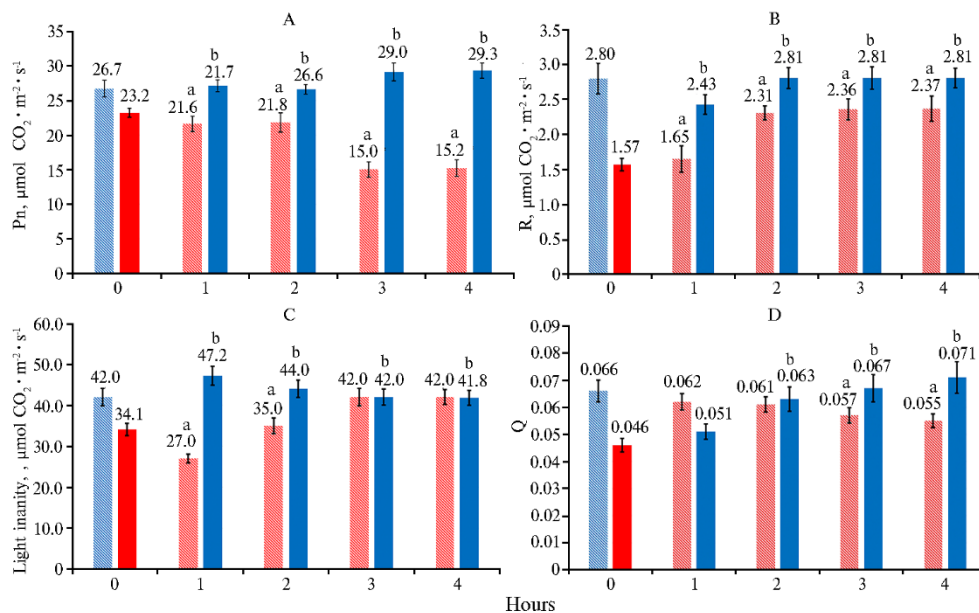


Fig. 3. The rate of photosynthesis on the plateau of the light curve (A), the rate of respiration (B), the light compensation point (C) and the quantum yield of photosynthesis (D) in the leaves of kok-saghyss (*Taraxacum kok-saghyss* E. Rodin) plants during long-term aeroponic cultivation in chambers of an aeroponic phytotron at a light intensity of $400 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a predominance of blue light (BS, left columns of the leftmost pair) or red light (RS, right columns of the leftmost pair), and when the irradiation spectrum changes from BS to RS (left bars of the remaining pairs) and from RS to BS (right bars of the remaining pairs) ($M \pm \text{SEM}$, $n = 6$).

^{a, b} The differences from the RS option at the starting point and from the BS option at the starting point, respectively, are statistically significant at $P = 0.95$.

When changing the irradiation mode from BS to RS, the rate of photo-

synthesis decreased from 26.7 to 15.2 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the plateau of the light curve (at light saturation) (Fig. 3, A). When switching from BS to RS, there was also a decrease in the rate of dark respiration from 2.80 to 2.38 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (see Fig. 3, B) and in the quantum yield of photosynthesis from 0.066 to 0.055 (see Fig. 3, D). Changing the light regime from RS to BS increased the rate of photosynthesis at the photosynthesis light curve saturation, the rate of dark respiration, the quantum yield of photosynthesis, and the light compensation point.

The observed changes in the activity of the photosynthetic apparatus may be associated with the activity of the light stage of photosynthesis. Thus, changing the regime from RS to BS initially led to a decrease in the maximum quantum yield of PSII, the effective quantum yield, and the electron transport rate, but non-photochemical fluorescence quenching increased (Fig. 4). After 3 hours, the values were comparable to those in plants irradiated predominantly with RS before changing the light regime.

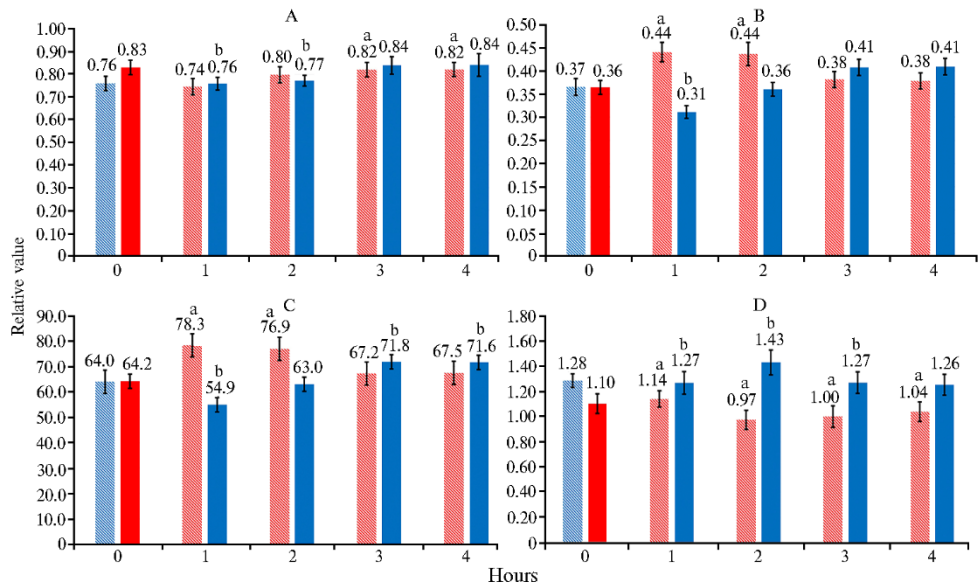


Fig. 4. Maximum quantum yield of PSII (A), effective quantum yield (B), electron transport rate (C) and non-photochemical quenching (D) in leaves of kok-saghyss (*Taraxacum kok-saghyss* E. Rodin) plants during long-term aeroponic cultivation in chambers of an aeroponic phytotron at a light intensity of 400 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a predominance of blue light (BS, left columns of the leftmost pair) or red light (RS, right columns of the leftmost pair) and when the irradiation spectrum changes from BS to RS (left bars of the remaining pairs) and from RS to BS (right bars of the remaining pairs) ($M \pm \text{SEM}$, $n = 6$).

^{a, b} The differences from the RS option at the starting point and from the BS option at the starting point, respectively, are statistically significant at $P = 0.95$.

Changing the spectral mode from BS to RS led to a gradual increase in the maximum quantum yield of PSII, an increase in the effective quantum yield and electron transport rate during the first 2 hours, and a decrease in non-photochemical fluorescence quenching. After 3 hours, the values were the same or lower (e.g., for non-photochemical quenching) compared to those for plants irradiated predominantly with BS before changing the light regime.

The mechanisms by which certain parts of the spectrum and light irradiation regimes affect the primary and secondary metabolism of plants differ. The influence of the irradiation spectrum when growing plants is manifested both in changes in the operation of the photosynthetic apparatus and in the direction of secondary metabolism reactions. Thus, a change in the irradiation spectrum of

plants from BS to RS led to an increase in the concentration of glucose and sucrose compared to the initial values. This dependence persisted for 2 hours, then the concentration of sucrose remained at the same level, and the glucose content decreased. When switching from RS to BS, the opposite effect occurred. At first, the concentrations of glucose and sucrose decreased and then increased (Fig. 5).

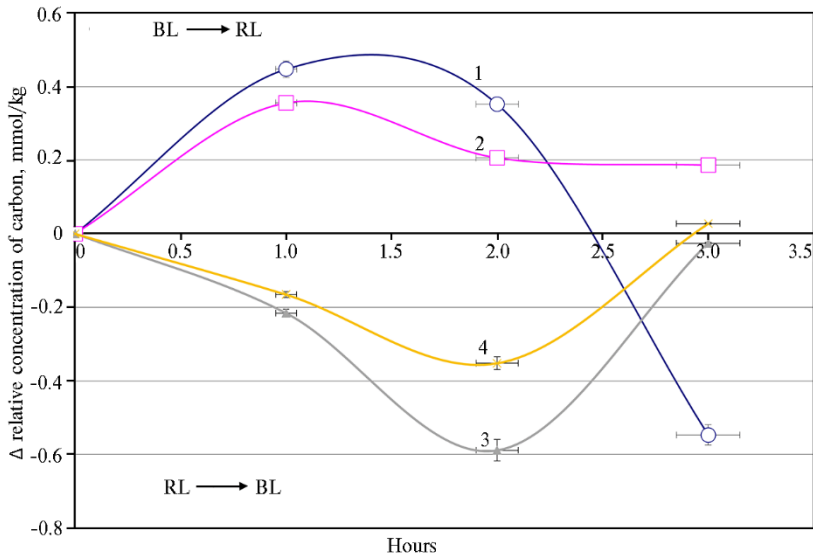


Fig. 5. Changes in the concentration of glucose (1, 3) and sucrose (2, 4) in the leaves of kok-saghyz (*Taraxacum kok-saghyz* E. Rodin) plants during long-term aeroponic cultivation in the chambers of an aeroponic phytotron at a light intensity of $400 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a predominance of red light (RS) or blue light (BL) after changing the irradiation spectrum from BS to RS (1, 2) and from RS to BS (3, 4) ($M \pm \text{SEM}$, $n = 8$).

Soluble carbohydrates are plant metabolic substrates important for various physiological and biochemical processes, regulating carbon transport and incorporation into metabolism. In plant leaves, about half of the fixed carbon is transported in the form of sucrose or glucose to the stem and roots where they are converted into polysaccharides, and in kok-saghyz mainly into inulin. Along with the accumulation of inulin, the synthesis and accumulation of isopentenyl pyrophosphate (IPP), a rubber monomer, occurs. Plants use two pathways for PPI biosynthesis, through mevalonate (MVA) and methylerythritol (MEP). The mevalonate pathway occurs in the cytosol of the cell, the methylerythritol pathway in the plastids. Isopentenyl pyrophosphate biosynthetic enzymes in both pathways convert intermediates generated from the metabolism of sucrose via pyruvate and glyceraldehyde-3-phosphate or via acetyl-CoA, respectively, for the MEP and MVA pathways [30, 31]. In the cytoplasmic MVA pathway, the main substrate is cytosolic acetyl-CoA, derived from either sucrose or glucose and fructose. Changing the biosynthesis of these products [32, 33] can be an effective lever for regulating the growth and accumulation of biomass by kok-saghyz plants, and ultimately the synthesis and accumulation of inulin and rubber.

Active accumulation of these compounds occurred in the roots of kok-saghyz plants grown in the chambers of an aeroponic phytotron for 28 days (Table). When plants were irradiated with a higher proportion of RS, there was an increase in the rubber and inulin contents by 3.0 and 4.1 times, respectively, with a higher proportion of BS, by 5.4 and 4.6 times compared to the initial values before planting in the phytotron. The effect of the spectral composition on day 28 was reliably expressed in an increase in the rubber content in plants grown under a higher proportion of blue light.

The content of rubber and inulin in the roots of kok-saghyz (*Taraxacum kok-saghyz* E. Rodin) plants during long-term aeroponic cultivation in the chambers of an aeroponic phytotron at a light intensity of 400 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a predominance of red light (RS) or blue light (BS) ($M \pm \text{SEM}$, $n = 5$)

Light intensity in phytotron	Time	Rubber, %	Инулин, %
400 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a larger proportion of RS in the irradiation spectrum	Planting in a phytotron	2.0 \pm 0.1	3.8 \pm 0.1
	28 days	5.9 \pm 0.2*	15.6 \pm 1.1*
400 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a larger proportion of BS in the irradiation spectrum	Planting in a phytotron	1.9 \pm 0.1	4.0 \pm 0.1
	28 days	10.3 \pm 0.3*	18.3 \pm 1.2*

* Differences from values at planting are statistically significant at $P = 0.95$.

The aeroponic phytotronic technologies under defined cultivation parameters (temperature, humidity, CO₂ content, light intensity and spectral composition, mineral nutrition, etc.) create a strategy for controlling plant metabolism depending on the growth stage and/or the feasibility of choosing a priority pathway for the directed biosynthesis of secondary metabolites, rubber and inulin.

According to our results, light with a predominance of red or blue components affects the biosynthesis of soluble sugars, rubber and inulin in kok-saghyz plants. An increase in rubber biosynthesis under the influence of BS may be associated with an adjustment in the metabolic pathways of carbohydrate metabolism due to the specific effect of BS on the photosynthetic apparatus.

As it was shown, an increase in the synthesis of soluble carbohydrates or proteins when plants are irradiated predominantly in the red or blue spectrum [34] causes a change in the rate of appearance and growth of new leaves, and in the ratio of the biomass of the above-ground parts of plants and the root system [35]. When plants are irradiated with BS, photosynthesis products are not used for rapid leaf growth, therefore, sucrose or glucose flow into the roots where, after a series of biochemical reactions in the cytoplasmic MVA pathway, secondary metabolic products are synthesized. In our experiments, when BS predominated in the radiation spectrum, a greater accumulation of rubber in the roots occurred compared to the RS predominance.

Along with long-term exposure of plants to RS or BS, periodic changes in the irradiation spectrum can also occur [36], which affects the rate of synthesis and the accumulation and transport of photosynthetic products. In our tests, a change in the spectral mode of irradiation of kok-saghyz plants for several hours from BS to RS did not cause a decrease in the rate of photosynthesis (see Fig. 2), however, there was an increase in the rate of electron transport and the real quantum yield, as well as a decrease in non-photochemical quenching of fluorescence. More efficient PSII operation increases the concentration of glucose and sucrose in the leaves.

When switching from RS to BS, the rate of photosynthesis sharply decreased within 1 hour, after which (after 2-3 hours) there was an increase in the rate of CO₂ absorption, a decrease in the quantum yield of PSII and the rate of electron transport. Non-photochemical quenching also increased at the very beginning of the transition from RS to BS, then, the values returned to its original figures. Under these conditions, a decrease in the concentration of glucose and sucrose in the leaves was noted during the first 2 hours, followed by a return to initial values after 3 hours (see Fig. 5).

A change in the activity of the photosynthetic apparatus in response to modulation of the spectral composition of irradiation develops over several hours, and this technique can be used to regulate the rate of metabolic processes and the yield of final products. A similar approach can also be used to increase the accumulation of products of secondary metabolism of kok-saghyz plants, primarily rubber. However, further research is required to understand the key factors influencing the biosynthesis of target substances, as well as the study of gene expression and protein synthesis involved in rubber biosynthesis. In combination

with genetic engineering methods for improving the productivity of rubber plants, this will significantly increase the yield of rubber and inulin to economically acceptable values. The comprehensive processing of all grown plant biomass to obtain various valuable products will undoubtedly play a major role in the commercialization of aeroponic cultivation of kok-saghyz.

Thus, changes in the light regime during long-term cultivation of kok-saghyz plants in the controlled conditions of the phytotron influenced the activity of light processes of the photosynthetic apparatus, i.e., the maximum and effective quantum yield, the rate of electron transport, and the rate of carbon dioxide absorption. An increase in the proportion of red light (RS) in the irradiation spectrum of plants grown under the predominance of blue light (BS) led to an increase in the rate of electron transport and effective quantum yield, and to a decrease in non-photochemical quenching of fluorescence after 3 hours. The data obtained indicate that photosynthetic apparatus can utilize red light more effectively. When switching from RS to BS, an increase in the rate of photosynthesis and respiration occurs. When changing the irradiation regime from BS to RS, on the contrary, the rate of photosynthesis decreased from 26.7 to 15.2 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (at light saturation), the rate of dark respiration decreased from 2.80 to 2.38 $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, quantum yield of photosynthesis from 0.066 to 0.055. Higher proportion of BS in the irradiation spectrum increases the accumulation of rubber in the kok-saghyz roots 1.75-fold compared to plants grown under irradiation with greater level of the RS. In addition, a 1.17-fold increase in inulin accumulation occurs. The kok-saghyz growing technology based on these effects seems very promising to ensure the biosynthesis of rubber and inulin under controlled phytotron conditions with the optimization of all growth factors.

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