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Changes in the optical properties of bean leaves during photosynthetic apparatus formation

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This study investigates the optical characteristics of bean leaves (*Vicia faba* L., cultivar „Russian black"), which reflect the functional properties of the photosynthetic apparatus (PSA) in chloroplasts. The functioning of the PSA was assessed based on the kinetics of chlorophyll *a* fluorescence induction and light-induced changes in absorption, reflecting the redox transformations of photosystem 1 reaction centers (P_{700}). A comparison was made between the optical properties of leaves from control samples (plants grown under moderate light intensity, 2000−3000 lx) and etiolated leaves from plants cultivated under low light (5−10 lx). Using chlorophyll *a* fluorescence and changes in the P_{700} state, we monitored the formation of the photosynthetic apparatus in chloroplasts during the illumination of etiolated leaves. It was shown that the development of active chloroplasts in etiolated leaves occurs within 2−3 days after exposure to intense light. An important feature of the diagnostic methods used in this study is their non-invasive nature, eliminating the need for chloroplast (or their component) isolation. Thus, instrumental analysis of leaf optical properties enables non-invasive monitoring of the plant PSA *in situ* at various stages of cultivation.

Keywords: plant photosynthetic apparatus, chlorophyll fluorescence, electron transport.

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Introduction

De-etiolation of plant leaves (or the transition from growth in the dark to photomorphogenesis) is one of the most difficult stages of plant ontogenesis. Phytohormones and light play an essential role in this process [1,2]. Chlorophyll is synthesized in the course of de-etiolation, which is the most important pigment of photosynthesis, and the photosynthetic apparatus is formed [3,4]. The photosynthetic activity of plants increases along with an increase in the chlorophyll content [5,6]. During this period of ontogenesis, regulatory mechanisms are formed that ensure the optimal operation of two plant photosystems and, ultimately, the entire photosynthetic apparatus [7,8]. The study of these regulatory mechanisms is an important and urgent task of plant physiology and biophysics of photosynthesis. Bean sprouts *Vicia faba* L. were grown in this study in shading conditions, and then they were transferred to the conditions of intense natural light. Within one week after such transfer, the chlorophyll content, the fluorescent characteristics of the green leaves, as well as the kinetic curves of the photoinduced redox transformations of the reaction centers of the photosystem 1 were recorded.

The fluorescent parameters of photosynthetic objects depend on a wide range of biotic and abiotic factors, they allow monitoring changes in the structural and functional organization of the photosynthetic apparatus (PSA) of plants [9–12]. Of particular interest are the induced changes in fluorescence parameters recorded using currently widely used pulsed fluorometers, which make it possible to determine a number of important parameters from the point of view of the functioning of the PSA [13,14]. Recording of the kinetics of changes in the state of P_{700} under the action of light of different spectral composition allows studying the features of the joint functioning of photosystems 1 and 2 [15,16]. It was assumed that the use of these spectroscopic research methods would provide additional information about the features of the formation of the photosynthetic apparatus of plants during de-etiolation.

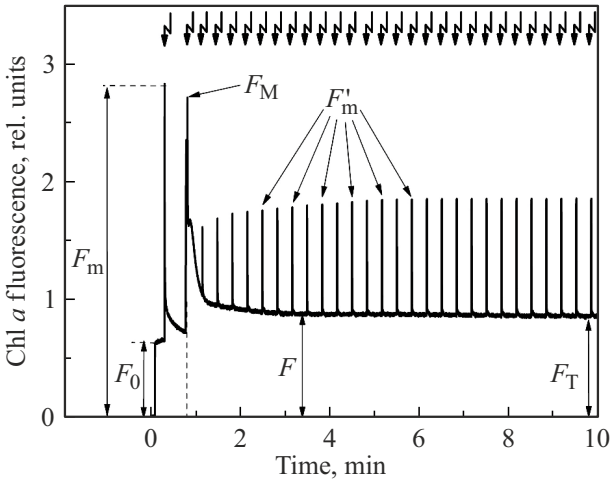

Figure 1. Protocol for fluorescence measurements with a PAM fluorometer. Zigzag arrows indicate the moments when saturating flashes of light are switched on.

1. Objects and methods

Bean sprouts *Vicia faba* L. of variety „Russian black“ were studied. The plants were grown in 0.5 L bags with soil at a temperature of 25°C in laboratory conditions with different lighting conditions: one option in shading conditions with illumination of 5–10 lx (experimental plants), and the other option with illumination of 2000–3000 lx (control plants). The duration of illumination of plants with natural light was 15 h per day. The illumination was measured in the daytime using a portable digital luxmeter Mastech MS6610. The experimental plants were transferred to high-light conditions after three weeks of growing. Immediately before the transfer, as well as daily for the next week, the chlorophyll content, the fluorescence parameters of the leaves of control and experimental plants, as well as the kinetics of light-induced oxidation of reaction centers P_{700} were measured.

The kinetics of changes in chlorophyll fluorescence yield in bean leaves was measured using a pulsed fluorometer PAM-2500 (Waltz, Germany). The leaf, without removing it from the stem, was placed in the device and was kept in the dark for 5 minutes before the start of measurements. The protocol for measuring the fluorescence intensity is shown in Fig. 1. Fluorescence was stimulated by pulsed measuring light ($\lambda = 630$ nm, $\Delta\lambda = 5$ nm, $I = 10\,\mu$E/(m^{2}s)); the initial fluorescence level F_0 was determined immediately after switching on the „measuring“ light. The measuring light comprises millisecond (5 ms) flashes of low intensity ($I = 10\,\mu$E/(m^{2}s)), supplied in periodic pauses when the stronger actinic light was turned off, the intensity of which significantly exceeded the intensity of the measuring light, ($I = 500\,\mu$E/(m^{2}s)). The essence of such a standard protocol for sample illumination is that „actinic“ light is used for light-induced changes in the state of the photosynthetic apparatus of plants, and fluorescence measurements were performed in response to flashes of „measuring“ light during short-term dark pauses when „actinic“ light was not applied. The low intensity of the measuring light practically does not change the state of the photosynthetic apparatus.

The light-harvesting complexes of the photosystem 2 (PS2) are the main source of chlorophyll fluorescence in plant leaves (80% and above) [11,17]. The maximum fluorescence level F_m was determined when the leaf was illuminated with a saturating flash of light ($\lambda = 630$ nm, $\tau = 0.5$ ms, $I = 3400\,\mu$E/(m^{2}s)). Induced changes in chlorophyll *a* fluorescence were recorded after switching on continuous actinic light ($\lambda = 455$ nm, $I = 500\,\mu$E/(m^{2}s)). Saturating flashes of light, applied against a background of continuous light, followed with an interval of 20 s. The ratio $F_V/F_m = (F_m - F_0)/F_m$ of photochemical transformations to PS2 and $\Phi_{PSII} = (F'_m - F)/F'_m$ (characterizes the effective quantum yield of photochemical transformations to PS2 at the time of applying a saturating flash of light) was determined as the fluorescence parameters. The parameter

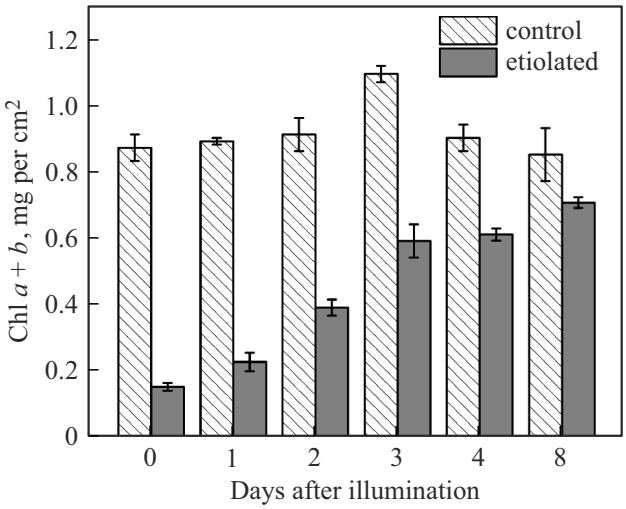

Figure 2. Dependence of the chlorophyll content (mg per 1 cm^2 of the leaf blade) on the time elapsed since the beginning of illumination of etiolated leaves (control plants are plants grown in natural light conditions).

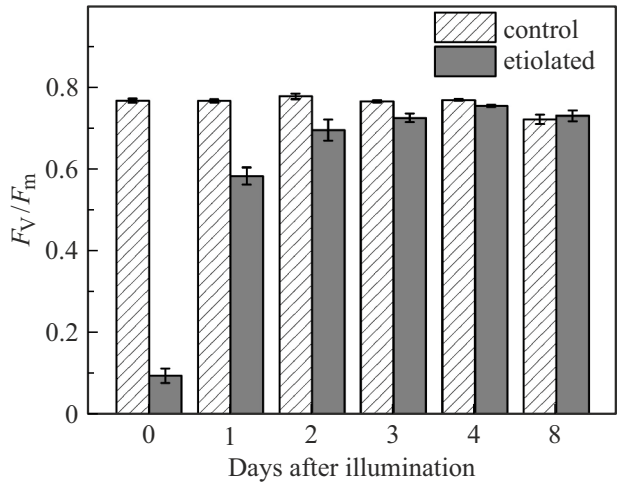

Figure 3. Dependence of the indicator $F_V/F_m = (F_m - F_0)/F_m$ on the time elapsed since the beginning of illumination of etiolated leaves (control plants are plants grown in natural light conditions).

$NPQ = (F_m - F'_m)/F'_m$ characterizes the coefficient of non-photochemical quenching of fluorescence.

The values of fluorescence parameters and chlorophyll content, averaged for a series of four samples, are given. Also, the sample standard deviation of the arithmetic mean was calculated for each indicator used.

The light-induced oxidation of the P_{700} centers in the leaves was judged by the difference in light absorption at wavelengths of 870 and 830 nm (optical signal $\Delta A_{870-830}$) according to the method described earlier [15].

The chlorophyll content in plant leaves was determined using acetone extracts according to the procedure described in Ref. [18].

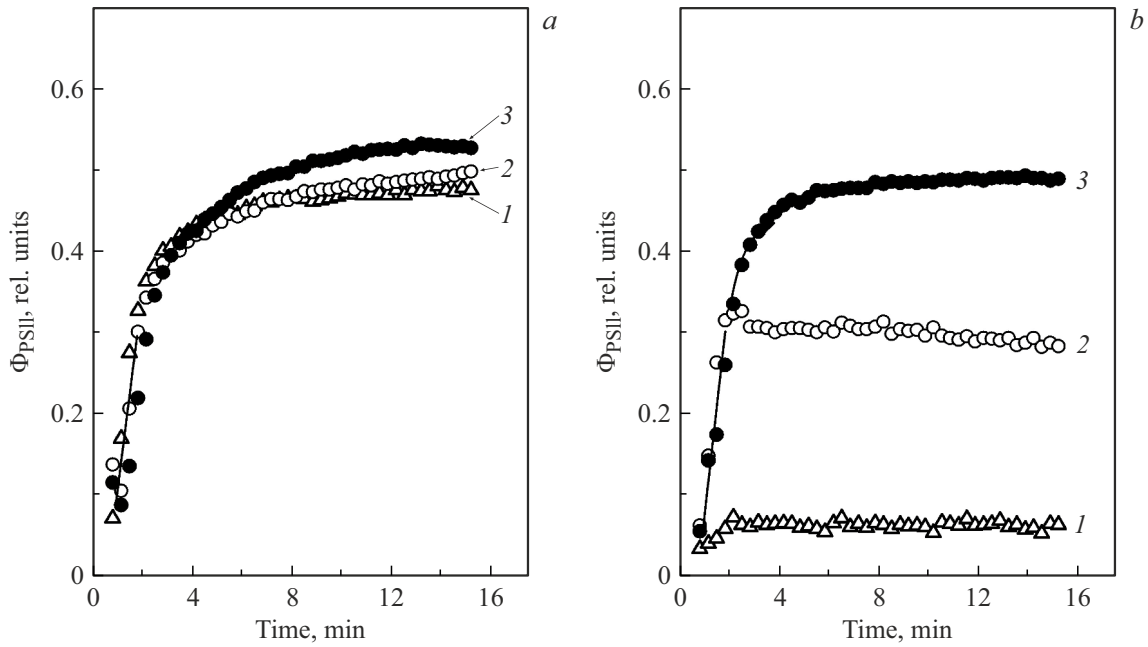

Figure 4. Characteristic curves of the indicator Φ_{PSII} of bean leaves grown in natural light (*a*) and in shading (*b*). The curves were recorded: immediately after moving the etiolated samples to natural light conditions (*1*), 1 day later (*2*), 4 days later (*3*).

2. Results and discussion

The etiolated bean seedlings grown under shading conditions differed significantly in appearance from those grown in the light. They had elongated stems and thin elongated leaves with pale green coloration and low chlorophyll content. After moving these seedlings to high-light conditions, the chlorophyll content gradually increased, both per plant biomass and per leaf blade area (Fig. 2). Along with an increase in the chlorophyll content, there was a gradual increase of $F_V/F_m = (F_m - F_0)/F_m$ — the ratio of variable fluorescence $F_m - F_0$ to the maximum F_m (Fig. 3). This indicator characterizes the efficiency of using light in PS2 [17,19] or, in other words, the maximum quantum yield of photochemical transformations in PS2 [10]. Indeed, the yield of chlorophyll fluorescence during the action of short-term measuring light flashes depends on the state of the PSA: it is minimal when the reaction centers (RC) are active („open“) and can capture excitation energy from the pigment matrix, and maximal when RC are inactive („closed“). Thus, an increase in the fluorescence yield at „closing“ RC characterizes that part of the light energy that was used in the primary photosynthetic reactions with „open“ RC [20]. The gradual increase in the indicator F_V/F_m in the first week after the start of illumination of etiolated seedlings reflects an increase in the activity of PS2, which, as is known, is formed later than PS1 [5].

The kinetic curves of the indicator $\Phi_{PSII} = (F'_m - F)/F'_m$, which characterizes changes in the quantum yield of photochemical transformations of PS2 after switching on the active light, indicate an increase in the activity of PS2 during de-etiolation of leaves (Fig. 4). The active (actinic) light has a sufficiently high intensity, which affects the processes of photosynthesis and changes the state of the photosynthetic apparatus. In control plants, the steady-state values of Φ_{PSII} remained virtually unchanged during the entire measurement period, while in experimental plants they increased monotonously, similar to the growth of the chlorophyll content and the indicator F_V/F_m (Fig. 5). It can be assumed that an increase in the values of Φ_{PSII} indicates an increase in photosynthetic activity in general. This is consistent with the data from Ref. [21], which assumes a proportional relationship between the quantum yield of fixation of CO_2 and steady-state values of Φ_{PSII}, observed on a number of objects under certain experimental conditions.

Quenching of chlorophyll *a* fluorescence as illuminated by continuously acting light (Fig. 1) is usually divided into photo- and non-photochemical quenching [9, 10, 22]. Photochemical quenching of fluorescence is associated with a change in the redox state of the electron acceptors PS2 [23]. The non-photochemical quenching of chlorophyll *a*, which is part of PS2, is associated, firstly, with the formation of a transmembrane difference in proton concentration (ΔpH) on the membrane (the so-called energy quenching) and, secondly, with the redistribution of the energy of absorbed light in favor of PS1 (transitions „state 1“–„state 2“) [9,10,24–26]. It is believed that such transitions are associated with the reversible phosphorylation of the mobile light-harvesting pigment-protein complex (LHCII) and its movement between membrane regions with different concentrations of complexes PS1 and PS2 [21,27,28]. The increase in fluorescence measured at the time of application of the saturation flash measuring light flashes, as well as the decreased values of the fluorescence level (F'_m), shown in

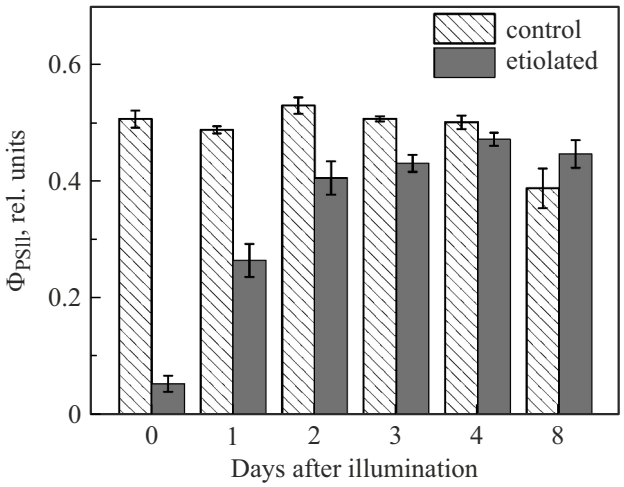

Figure 5. Dependence of the indicator $\Phi_{PSII} = (F'_m - F)/F'_m$ on the time elapsed since the beginning of illumination of etiolated leaves (control plants are plants grown in natural light conditions).

Fig. 1, reflect phenomena such as photochemical and non-photochemical quenching of chlorophyll *a* fluorescence (see details in Ref. [15]).

Prior to the 4th day of de-etiolation, the kinetic curves for *NPQ* (coefficient of non-photochemical quenching of fluorescence) had a pronounced anomalous character, indicating an insufficiently developed photosynthetic apparatus: lowered values of *NPQ* and a delayed (more than 15 min) achievement of a stationary level (Fig. 6). The nonmonotonic character of kinetic curves is assumed to be related to the formation of ΔpH on the thylakoid membrane at the beginning of illumination and the subsequent decrease in ΔpH due to the progressive synthesis of ATP [29–31]. The steady-state values of the parameters *NPQ* and Φ_{PSII} gradually increase as the leaves de-etiolated, reflecting an increase in photosynthetic activity of seedlings and the formation of regulatory mechanisms that optimize photosynthetic processes.

In addition to fluorescent methods for noninvasive diagnosis of PSA formation, optical methods based on measurements of light absorption by light-harvesting pigments that are part of PSA can be used. As an example, let us consider experimental data on measurements of the kinetics of light-induced oxidation of reaction centers P_{700}. The oxidation of P_{700} was judged by measurements of the difference signal $\Delta A_{870-830}$, which is the difference in light absorption measured at wavelengths of 870 and 830 nm (see details [15]). The amplitude of the signal $\Delta A_{870-830}$ is proportional to the number of oxidized centers P_{700^+}. Fig. 7, *a* shows a typical curve of light-induced changes in the magnitude of the difference signal $\Delta A_{870-830}$ in the control sample after switching on continuous red light ($\lambda_{max} = 635$ nm, light λ_{635}), effectively exciting both photosystems, and high-beam red light ($\lambda_{max} = 720$ nm, light λ_{720}), exciting mainly PS1. In leaves adapted to darkness for 5 min, in response to the inclusion of red light, the signal $\Delta A_{870-830}$ increases to a steady level due to photooxidation of the centers of P_{700}. The signal growth occurs after a certain delay, which is explained by the fact that after the leaf adapts to darkness, the Calvin–Benson cycle (CBC) is inactive. In this case, the outflow of electrons from PS1 is limited due to the low consumption of NADPH which is the reduced final electron acceptor in PS1 [32]. As the chloroplasts are illuminated with red light (λ_{635}), CBC enzymes are activated, the rate of electron outflow from PS1 increases, resulting in noticeable oxidation of P_{700}. After turning off the light, a relatively rapid recovery of P_{700^+} is observed (the half-life of the signal from P_{700^+} is $t_{1/2} \sim 20-30$ ms). This is attributable to reduced plastoquinol molecules (PQH_2) formed during the exposure to light λ_{635}, which excites PS2. The subsequent activation of the far-red light (FRL, λ_{720}), which mainly excites PS1, leads to a relatively rapid increase in the signal from P_{700^+}. At the same time, the decay of the signal $\Delta A_{870-830}$ slows down in the dark ($t_{1/2} \sim 1-3$ s), since PS2 is practically not excited under the exposure to FRL, and plastoquinone pool molecules are not restored.

A different pattern is observed in the case of etiolated leaves (Fig. 7, *b*). The red-light-induced signal $\Delta A_{870-830}$, which can be attributed to the oxidized centers P_{700^+}, is noticeably lower than in the control samples (Fig. 7, *a*). In response to the activation of the far-red light, which mainly excites PS2, the signal value is noticeably lower than in the leaves of control plants. These differences are explained by the fact that the reaction centers of PS1 have not yet formed in the etiolated leaves. The signal indicating the formation of PS1 appears only after a sufficiently long exposure of plants to light conditions (several days).

Conclusion

This paper used the example of bean leaves (*Vicia faba* L., variety „Russian black“) to show how it is possible to diagnose the state of PSA and monitor the formation of active chloroplasts *in situ* during leaf de-etiolation based on the optical parameters of plant leaves, measured by the kinetics of chlorophyll *a* fluorescence yield and light absorption by photoreaction centers P_{700}. An important feature of these diagnostic methods is that they are non-invasive: to assess the functional state of PSA, it is not necessary to isolate chloroplasts (or their components) from the leaves. Thus, using instruments based on the optical properties of leaves, it is possible to carry out long-term monitoring of the functioning of plant PSA *in situ* at various stages of their cultivation.

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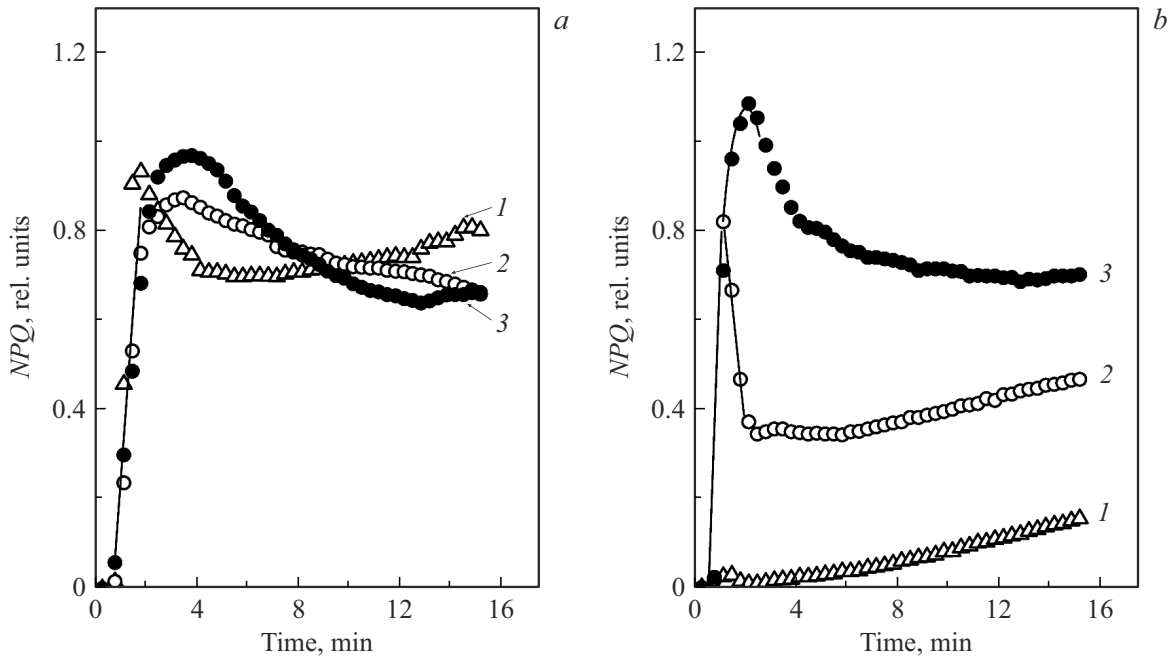

Figure 6. Characteristic curves of the indicator NPQ of bean leaves grown in natural light (*a*) and in shading (*b*). The curves were recorded: immediately after moving the etiolated samples to natural light conditions (*1*), 1 day later (*2*), 4 days later (*3*).

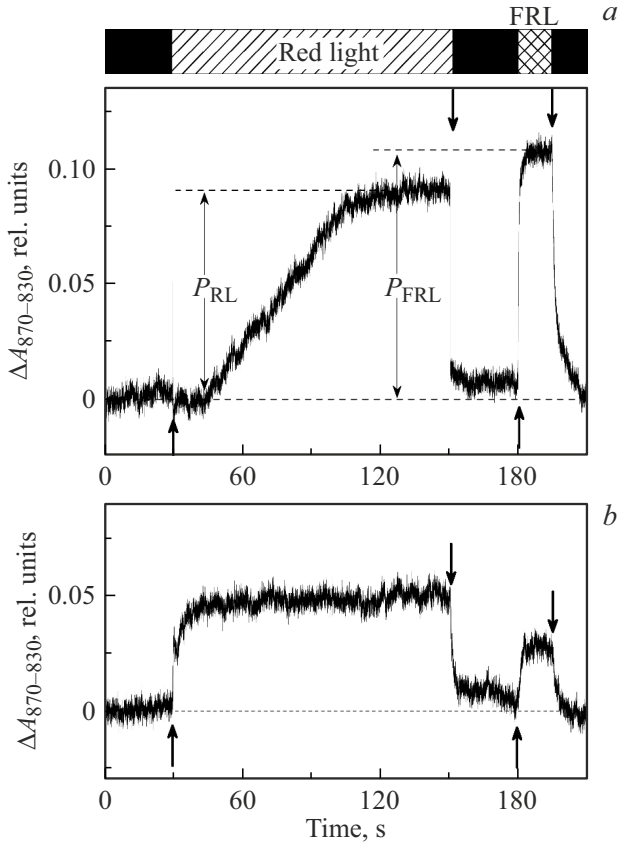

Figure 7. Typical curves of light-induced changes in the magnitude of the difference light absorption signal measured at 870 and 830nm (signal $\Delta A_{870-830}$) in the control sample (*a*) and the etiolated leave (*b*).

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Conflict of interest

The authors declare no conflict of interest.

References

[1] V.V. Kuznetsov, N.V. Kudryakova, M.N. Danilova. Fiziologiya rasteniy, **67** (6), 563 (2020) (in Russian). DOI: 10.31857/S001533032006010X

[2] G.E. Edwards, D.A. Walker. *C_3, C_4, mechanisms, and cellular and environmental regulation of photosynthesis* (Blackwell Scientific Publications, 1983).

[3] S.V. Konev, I.D. Volotovsky. *Photobiology*, izd. 2-e (BGU, Minsk, 1979) (in Russian).

[4] B.B. Buckanan. Ann. Rev. Plant Physiol., **31**, 341 (1980). DOI: 10.1146/annurev.pp.31.060180.002013

[5] V.F. Gavrilenko, M.V. Gusev, K.A. Nikitina, P. Hoffmann. *Izbrannye glavy fiziologii rastenij* (MGU, M., 1986) (in Russian).

[6] R.E. Blankenship. *Molecular mechanisms of photosynthesis* (Blackwell Science Inc., Malden, MA, 2002).

[7] S. Matsubara, B. Förster, M. Waterman, S.A. Robinson, B.J. Pogson, B. Gunning, B. Osmond. Phil. Trans. R. Soc. B: Biol. Sciences, **367** (1608), 3503 (2012). DOI: 10.1098/rstb.2012.0072

[8] Z. Li, S. Wakao, B.B. Fischer, K.K. Niyogi. Ann. Rev. Plant Biol., **60** (1), 239 (2009). DOI: 10.1146/annurev.arplant.58.032806.103844

[9] G.H. Krause, E. Weis. Annu. Rev. Plant Physiol. Plant Molec. Biol., **42**, 313 (1991).

DOI: 10.1146/annurev.pp.42.060191.001525
[10] D. Lazar. Biochim. Biophys. Acta, **1412** (1), 1 (1999). DOI: 10.1016/s0005-2728(99)00047-x
[11] *Chlorophyll a fluorescence: a signature of photosynthesis*, ed. by G.C. Papageorgiou (Springer, Dordrecht, 2004). DOI: 10.1007/978-1-4020-3218-9
[12] N.R. Baker. Annu. Rev. Plant Biol., **59**, 89 (2008). DOI: 10.1146/annurev.arplant.59.032607.092759
[13] K. Maxwell, G.N. Johnson. Exp. Botany, **51** (345), 659 (2000). DOI: 10.1093/jexbot/51.345.659
[14] B. Demmig-Adams, C.M. Cohu, O. Muller, W.W. Adams. Photosynth. Res., **113** (1–3), 75 (2012). DOI: 10.1007/s11120-012-9761-6
[15] I.S. Suslichenko, B.V. Trubitsin, A.V. Vershubskii, A.N. Tikhonov. Plant Physiol. Biochem., **185**, 233 (2022). DOI: 10.1016/j.plaphy.2022.06.002
[16] M.A. Benkov, I.S. Suslichenko, B.V. Trubitsin, A.N. Tikhonov. Biol. membrany., **40** (3), 172 (2023) (in Russian). DOI: 10.31857/S0233475523030039
[17] H.M. Kalaji, G. Schansker, M. Bresic et al. Photosynth. Res., **132**, 13 (2017). DOI: 10.1007/s11120-016-0318-y
[18] *Malyj praktikum po fiziologii rastenij*, pod red. M.V. Guseva (MGU, M., 1982) (in Russian).
[19] P.S. Venediktov, S.L. Volgin, Yu.V. Kazimirko i dr. Biofizika, **44**, 1037 (1999) (in Russian).
[20] B. Genty, J.-M. Briantais, N.R. Baker. Biochim. Biophys. Acta, **990**, 87 (1989). DOI: 10.1016/S0304-4165(89)80016-9
[21] J.F. Allen. Biochim. Biophys. Acta, **1098**, 275 (1992). DOI: 10.1016/s0005-2728(09)91014-3
[22] A.N. Tikhonov. Photosynth. Res., **116**, 511 (2013). DOI: 10.1007/s11120-013-9845-y
[23] G. Schansker, S. Tóth, A.R. Holzwart, G. Garab. Photosynth. Res., **128**, 43 (2014). DOI: 10.1007/s11120-013-9806-5
[24] E.H. Murchie, A.V. Ruban. The Plant J., **101**, 885 (2020). DOI:10.1111/tpj.14601
[25] J. Zaks, K. Amarnath, E.J. Sylak-Glassman, G.R. Fleming. Photosynth. Res., **116**, 389 (2013). DOI: 10.1007/s11120-013-9857-7
[26] X.-P. Li, A.M. Gilmore, S. Caffarri, R. Bassi, T. Golan, D. Kramer, K.K. Niyogi. J. Biol. Chem., **279** (22), 22866 (2004). DOI: 10.1074/jbc.M402461200
[27] J. Minagava. Biochim. Biophys. Acta, **1807**, 897 (2011). DOI: 10.1016/j.bbabio.2010.11.005
[28] M. Tikkanen, E.-M. Aro. Biochim. Biophys. Acta, **1817**, 232 (2012). DOI: 10.1016/j.bbabio.2011.05.005
[29] L.A. Malone, M.S. Proctor, A. Hitchcock, C.N. Hunter, M.P. Johnson. Biochim. Biophys. Acta., **1862** (5), 148380 (2021). DOI: 10.1016/j.bbabio.2021.148380
[30] A.N. Tikhonov. Biokhimiya, 88 (10), **1742** (2023) (in Russian). DOI: 10.31857/S0320972523100032
[31] A.N. Tikhonov. Photosynth. Res., **159**, 203 (2024). DOI: 10.1007/s11120-023-01034-w
[32] M. Kono, I. Terashima. J. Photochem. Photobiol. B: Biology, **137**, 89 (2014). DOI: 10.1016/j.jphotobiol.2014.02.016

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