ISSN 0006-3509, Biophysics, 2022, Vol. 67, No. 3, pp. 413–418. © Pleiades Publishing, Inc., 2022.
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CELL BIOPHYSICS

Thermoluminescence and Diagnostics of the State of Photosynthetic Apparatus of Plant Leaves

V. A. Karavaev[a], *, O. A. Kalmatskaya[a], B. V. Trubitsin[a], and A. N. Tikhonov[a], **

[a] *Faculty of Physics, Moscow State University, Moscow, 119991 Russia*

**e-mail: karavaev@phys.msu.ru*

***e-mail: an_tikhonov@mail.ru*

Received March 22, 2022; revised March 22, 2022; accepted March 28, 2022

Abstract—The basic mechanisms of the appearance of thermoluminescence bands of photosynthetic objects in the temperature range from −20°C to 80°C are considered. Examples are given to illustrate how the thermoluminescence method can be used for the diagnosis of the functional state of the photosynthetic apparatus of plants.

Keywords: thermoluminescense, oxygenic photosynthesis, electron transport

DOI: 10.1134/S0006350922030071

The phenomenon of thermoluminescence (TL) has found wide application in studies of physicochemical properties of various systems in physics, biophysics, geochemistry and paleontology. This phenomenon is widely used to study the energy structure of crystal phosphors when luminescent substances are created, to measure the absorbed dose of radiation, to diagnose the functional state of photosynthetic systems, and to analyze the properties of various minerals and archaeological artifacts [1, 2].

Studies of TL of biological objects began in the mid-1960s at the Department of Biophysics of the Faculty of Physics of Lomonosov Moscow State University. These studies were initiated by Professor L.A. Blumenfeld. Their premise was the hypothesis that light-induced processes of migration and conversion of energy in photosynthetic systems can proceed like semiconductor mechanisms of energy conversion in physical systems. Studies of biological systems by the TL method have been actively conducted at the Department of Biophysics for several decades by A.K. Kukushkin, M.K. Solntsev and their students. M.K. Solntsev designed a highly sensitive experimental setup that allowed relatively weak glow that occurs when pre-irradiated objects are heated to be recorded [3]. At the first stage, most attention was paid to the measurements of TL of powders of nitrogenous bases (adenine), nucleotides, and nucleic acids. These studies have shown the existence of processes of excitation energy migration between nucleotides, presumably protecting nucleic acids from radiation damage. Subsequently, the main research was focused on the study of photosynthetic objects (leaves and isolated chloroplasts). In these studies, optimal protocols of TL recording were developed that allow the functional state of the photosynthetic apparatus of plants growing under different conditions and exposed to different physiologically active compounds to be monitored. A significant contribution to the study of photosynthetic systems was made by the staff of the Department of Biophysics of the Faculty of Biology of Lomonosov Moscow State University [4].

In this article, we briefly consider the basics of the TL phenomenon and give examples of how the TL method can be used to diagnose the functional state of the photosynthetic apparatus of plants.

FUNDAMENTALS OF THE PHENOMENON OF THERMOLUMINESCENCE

Thermoluminescence is a glow that occurs when pre-cooled objects are heated and illuminated at a low temperature. When a substance is illuminated, carriers of opposite charges (electrons and "holes") may appear in it, which are localized and stabilized at the capture centers ("traps"). Recombination of opposite charges may be accompanied by a glow. For the release of electrons and "holes" from "traps", additional energy is needed, which can be communicated either by heating the object or by illuminating it with infrared light [5, 6]. If a pre-illuminated object is heated in the dark and the temperature at which the thermal energy becomes comparable to the activation energy (the energy needed to release the charge carrier from the trap) is reached, the substance begins to glow. Over

Abbreviations: TL, thermoluminescence; PS II, photosystem II; SFI, slow fluorescence induction.

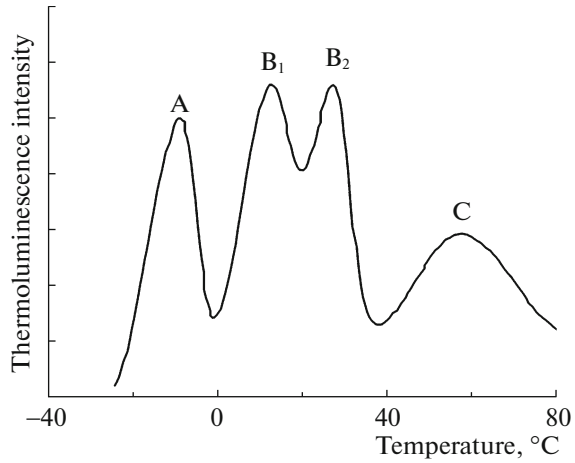

Fig. 1. Schematic representation of characteristic thermoluminescence bands of photosynthetic objects.

time, as the sample heats up, all electron-hole pairs recombine and the glow stops. By recording the dependence of the intensity of the glow on the temperature at which the radiation occurs, a TL curve is obtained, which carries information about the nature and energy characteristics of charge carrier traps.

STUDIES OF PHOTOSYNTHETIC SYSTEMS BY THERMOLUMINESCENCE

The nature of thermoluminescence spectra of chloroplasts of higher plants. The phenomenon of TL of photosynthetic objects was first observed by V. Arnold and N. Sherwood [7]. They recorded high-temperature thermochemiluminescence, which is usually associated with lipid peroxidation and which does not relate directly to photosynthesis. The components associated with photosynthesis were discovered later (see, for example, [8, 9]). The main source of the glow emitted by chloroplasts, the energy-transforming organelles of a plant cell, are excited chlorophyll molecules. After cooling of the sample and its illumination at low temperature and subsequent heating, the processes of reverse transfer of "holes" from the oxygen-releasing system and electrons from the quinone acceptors of photosystem II (PS II) occur; their recombination is accompanied by emission of stored energy [10, 11].

When the reaction centers are excited, a rapid separation of charges occurs with the transfer of an electron to the primary acceptor pheophytin. Next, the electron is transferred to the primary quinone acceptor Q_A, and then to the two-electron secondary quinone acceptor Q_B. The electron donor for the oxidized reaction center of PS II (P^+_{680}) is, ultimately, an oxygen-releasing system. The oxygen-releasing system can be in one of five states: S_0, S_1, S_2, S_3 and S_4. These states correspond to the states of manganese ions of varying degrees of oxidation. As a result of four-fold interaction with manganese ions, two water molecules decompose.

The oxygen molecule is released during the $S_4 \rightarrow S_0$ transition, for which light is not required.

In the studies of Solntsev et al. [12–16], TL of plant leaves was recorded mainly after their cooling and irradiation at –30°C. At this temperature, the oxidation processes of the secondary quinone acceptor Q_B^-, as well as the $S_4 \rightarrow S_0$ transitions, are "frozen", and short-term illumination of the sample with white light leads to the accumulation of electrons on the acceptor side of PS II and holes in its donor part. After further freezing to liquid nitrogen temperatures (77 K) and subsequent heating, three more or less pronounced bands with maxima in the ranges from –20 to 0°C (band A), from 0 to 40°C (band B, consisting of two components B_1 and B_2) and from 40 to 60–80°C (band C) were usually observed [17–20] (Fig. 1). At present, it can be considered established that bands A and B are directly related to the functioning of the photosynthetic electron transport chain [6]. It is assumed that band A arises mainly as a result of recombination of $S_3Q_A^-$ states [12], and band B as a result of recombination of $S_3Q_B^-$ and $S_2Q_B^-$ states [6]. It was, however, assumed that band A has a two-component composition; its low-temperature component is caused by the recombination of $S_4Q_A^-$ states, and the high-temperature component is caused by the recombination of $S_3Q_A^-$ states [13]. There is also a peak Q (herbicidal, or, in other designations, G-peak), observed in the range from 2 to 10°C when electron transfer from Q_A to Q_B is blocked; it was assumed that this band is mainly associated with the recombination of $S_2Q_A^-$ states [6].

Band B consists of two components, B_1 and B_2. The separation of these components occurs if the pH of the chloroplast suspension is less than 6.0; in the pH range 7.0–7.5, these two components are observed at the same temperatures and manifest themselves as one peak B [10]. It was assumed that the recombination of $S_3Q_B^-$ states was responsible for peak B_1, and the recombination of $S_2Q_B^-$ states was responsible for peak B_2 [2]. With a further increase in pH (higher than 8.0), peak B decreased. This was due to the precipitation of Mn ions associated with the oxygen-releasing system, which led to the suppression of oxygen release. This process was reversible by either reduction of pH or addition of NaCl [11].

The high-temperature band C is not directly related to photosynthesis. It is believed that it is caused by chemiluminescence accompanying the reactions of products that are formed as a result of the destruction of chloroplast membranes during freezing [14]. The

presence or absence of the C band is a test for the resistance of plants to certain adverse environmental factors: the less intense the C band, the wider the range of plant resistance to adverse conditions.

The effects of the physiological state of plants on the thermoluminescence spectra. The TL characteristics of leaves and chloroplasts are very sensitive to changes in the physiological state of plants [2]. Consider, for example, how the TL spectra of wheat leaves change with various mineral fertilizing of plants, which has been studied in detail [15]. In these experiments, plants were grown in vessels with soil, and they were watered with either tap water or water with the addition of nitrogen-containing, potassium, or phosphorus salts. With all types of fertilizers, an increase in the S_B/S_{total} index was observed, where S_B is the light sum (area under the curve) of TL in the temperature range from 0 to 40°C, and S_{total} is the light sum of TL in the range from 0 to 80°C. This effect was explained, on the one hand, by an increase in the number of Q_B^- accumulated by the start of TL recording, and, on the other hand, by a decrease in the light sum of band C. Band A was absent in these experiments. Obviously, this was due to the preliminary illumination of the sample at 0°C, when the oxidation processes of Q_B^- and $S_4 \rightarrow S_0$ transitions were not yet "frozen".

In a number of studies performed at the Faculty of Physics of Lomonosov Moscow State University, the TL of plant leaves under conditions of various photosynthetic activity was explored. In experiments with wheat plants affected by powdery mildew, it was found that changes in photosynthetic activity (the rate of O_2 release in the light) positively correlated with changes in the S_A/S_{total} index, where S_A is the light sum (area under the TL curve) of peak A, and S_{total} is the total light sum of TL (illumination of samples in these experiments was carried out at −30°C) [16]. Similar correlative relationships were found in the experiments with the treatment of plants with various physiologically active substances, and in conditions of both increased and decreased photosynthetic activity [17].

In another series of experiments performed by M.K. Solntsev et al., significant changes in the intensity of TL in the area of band A were observed in plants grown under conditions of fertilization with Kemira Lux fertilizer. In these experiments, wheat seeds were planted in sand bags with a volume of about 0.5 L. Before planting the seeds, the sand was treated until completely wetted with either water or solutions of Kemira Lux fertilizer (manufactured by Kemira Agro, Moscow oblast) in the proportion of 1 g fertilizer per 200 mL of water. The plants were grown in laboratory conditions, and TL measurements were carried out three weeks after planting. According to the instructions, Kemira Lux fertilizer contains the following components: 32% nitrogen, 20.6% phosphorus, 27.1% potassium, 0.1% iron, 0.02% boron, 0.01% copper, 0.1% manganese, 0.002% molybdenum, and 0.01% zinc. The use of Kemira Lux fertilizer led to a significant "flare-up" of peak A (Fig. 2), which was thought to be associated with an increase in the photosynthetic activity of wheat seedlings under conditions of mineral fertilization. At the same time, in this case, there was a slight increase in the intensity of high-temperature TL (above 50°C), in the region of the C band, which obviously indicated some deterioration in the structural and functional characteristics of chloroplast membranes. This example clearly illustrates the possibilities of using the TL method to select optimal fertilizer consumption rates from the point of view of the effect on the photosynthetic apparatus of plants.

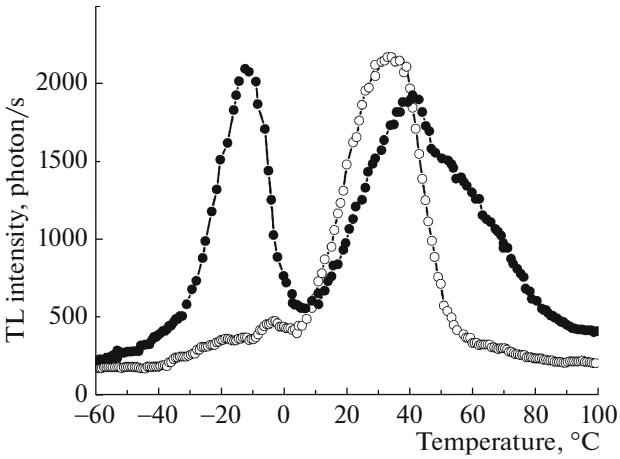

Fig. 2. Characteristic thermoluminescence curves of the wheat seedlings of the control plants (light circles) and plants treatment with the preparation "Kemira Lux" (dark circles).

In a number of studies by Karavaev, Solntsev, et al. [18–21], a comprehensive analysis of the luminescent indicators of plants treated with various physiologically active substances was carried out. The authors used indicators such as the relative light sums of TL (parameters S_A/S_{total} and S_C/S_{total}), as well as the values of relative fluorescence quenching $(F_M - F_T)/F_T$ when slow fluorescence induction (SFI) of chlorophyll *a* of photosynthetic objects was recorded. In experiments with bean seedlings treated with β-aminobutyric acid, a positive correlation was found between changes in the index $(F_M - F_T)/F_T$ of SFI of plant leaves, on the one hand, and the relative light sum of the A band of thermoluminescence, on the other. Along with this, a decrease in the contribution of the C band to the total light sum of TL was observed, which indicated a positive effect of the compound on the characteristics of the membranes [18].

In experiments with cucumber plants affected by thrips and treated with amaranthine (a nitrogen-con-

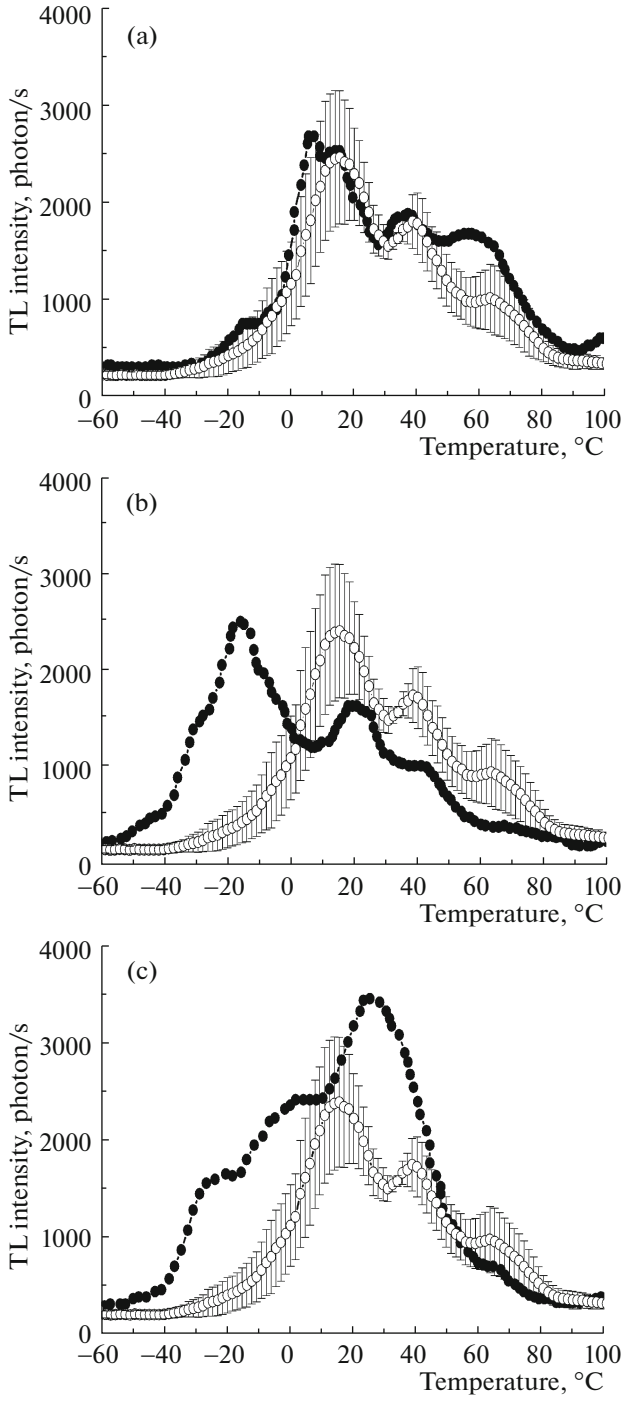

Fig. 3. Characteristic thermoluminescence curves of the bean leaves of the control plants (light circles) and plants treated with supercritical fluid extracts of *Reynoutria sachalinensis* (dark circles). In the preparation of supercritical fluid extracts, CO_2 with 10% ethanol (a), CO_2 with 2% ethanol (b) and pure CO_2 (c) were used.

taining alkaloid contained in amaranth leaves and inflorescences), a decrease in the values of $(F_M - F_T)/F_T$ of SFI of cucumber leaves affected by thrips (by 30–35% relative to the control) was recorded, which indicated a decrease in the specific (per chlorophyll) photosynthetic activity of the affected leaves. After treating the leaves with amaranth, the values of $(F_M - F_T)/F_T$ increased (up to 75–80% of the control), that is, photosynthetic activity was partially restored. TL measurements showed that when plants were affected by thrips, the intensity of the C band increases sharply at temperatures from 40°C to 80°C, and after treatment with amaranth, this intensity decreased. As noted above, the C band is caused by the chemiluminescence of products formed as a result of the destruction of membranes during freezing [14]. Based on these data, it was concluded that the resistance of thylakoid membranes to stressful effects caused by thrips decreased, and after treatment with amaranthine, it was partially restored [19].

The parameters of SFI and TL of the leaves of lilac *Syringa vulgaris* and silver maple *Acer saccarinum*, the cuttings of which were treated with indolyl butyric acid, zircon, kornevin and ribav-extra preparation before planting, were measured [20]. In the experiments with lilac seedlings, an increase in the values of $(F_M - F_T)/F_T$ of SFI and S_A/S_{total} TL was recorded compared to the control in the sequence indolylbutyric acid → kornevin → zircon, and in the experiments with maple seedlings in the sequence ribav-extra → kornevin → zircon → indolylbutyric acid. There was a decrease in the values of S_C/S_{total} in the same sequences. The results obtained indicated a positive effect of the treatment of cuttings with the studied preparations on the physiological state of lilac and maple seedlings.

When bean seedlings were treated with supercritical fluid extracts of the Sakhalin knotweed *Reynoutria sachalinensis* using carbon dioxide, an increase in the intensity of TL was observed in the region of negative temperatures (band A), but only if small concentrations of ethanol (2%) were used to obtain the extract or if ethanol was not used at all (Fig. 3) [21]. Calculations have shown that in both of these cases the relative light sum of the A band increased significantly (the S_A/S_{total} indicator, where S_A is the area under the TL curve in the range from –40 to 0°C and S_{total} is the area under the entire TL curve), which indicated an increase in the photosynthetic activity of plants. The most pronounced effect of the increase in S_A/S_{total} was expressed when 2% ethanol was used in the preparation of supercritical fluid extracts, which was consistent with the data obtained by the SFI method. In addition, in the variants with pure CO_2 and CO_2 with the addition of 2% ethanol, a decrease in high-temperature TL was observed in the C band region, which indicated an increase in the resistance of chloroplast membranes to adverse conditions [14]. At a high (10%) concentration of ethanol used as a co-solvent in the preparation of the extract, the TL intensity in the C band region significantly increased, which indicated

the negative impact of large amounts of ethanol on the structural and functional characteristics of chloroplast membranes. The stimulating effect of extracts from *R. sachalinensi*s on the photosynthetic apparatus of bean leaves might be associated with the entry of physiologically active compounds of quinone nature into the leaf cells that increased the pool of electron acceptors of PS II.

In a number of studies performed by M.K. Solntsev et al., the TL method was used to investigate the mechanisms of action of a number of drugs with herbicidal and fungitoxic effects on the photosynthetic apparatus of plants [17, 22, 23]. A large number of "photosynthetic" herbicides are inhibitors of electron transport between PS II and PS I. Changes in the state of the acceptors Q_A and Q_B during treatment with these herbicides led to corresponding changes in the TL curves. These studies have clearly demonstrated the possibilities of the TL method for evaluating the inhibitory effect of herbicides on primary photosynthesis processes. As noted in [24], the TL method compares favorably with an expensive and time-consuming method based on the measurements of radioactivity of appropriately labeled compounds.

A comparative study of the photosynthetic characteristics of tradescantia leaves grown under low (50–125 μE/(m^2 s) and high (875–1000 μE/(m^2 s) illumination conditions was carried out using TL, PAM-fluorimetry and electron paramagnetic resonance [25]. Significant differences were revealed in the ratio of light sum bands of TL with maxima at about 0 and 25–30°C. A conclusion was made about the differences in the pools of plastoquinone molecules between photosystems. It was assumed that the increased number of plastoquinone molecules on the acceptor side of PS II contributed to the effective course of photosynthesis in the leaves of plants grown at low illumination conditions.

Thus, extensive literature data, as well as long-term studies carried out at the Department of Biophysics of the Faculty of Physics of Moscow State University indicate the possibility of using the TL method to assess the functional activity of the photosynthetic apparatus of plants under various physiological conditions.

ACKNOWLEDGMENTS

This article is dedicated to the 100th anniversary of the birth of our teacher, Professor L.A. Blumenfeld, who initiated TL research at the Department of Biophysics of the Faculty of Physics of Lomonosov Moscow State University. The main contribution to the implementation of the program of various studies in this area was made by our friend and colleague Mikhail Konstantinovich Solntsev. He left us the installation he created for studying biological objects by the method of TL and detailed protocols for studies of photosynthetic objects. M.K. Solntsev's scientific discoveries in the field of practical applications of the TL method in agrophysics and biophysics of photosynthesis remain relevant. The bright memory of M.K. Solntsev, to whom we dedicate this article, is unquenchable. The authors express their gratitude to the professor of the Department of Biophysics of the Faculty of Physics of Lomonosov Moscow State University V.I. Lobyshev for valuable recommendations on writing the article.

FUNDING

The work was carried out with the financial support of the Russian Science Foundation, project no. 21-74-20047.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals or human subjects performed by any of the authors.

REFERENCES

1. K. K. Shvarts, Z. A. Grant, T. K. Mezhs, et al., *Thermoluminescent dosimetry* (Zinatne, Riga, 1968).
2. Y. Inoue and K. Shibata, "Thermoluminescence from photosynthetic apparatus," in *Photosynthesis: Energy Conversion by Plants and Bacteria*, Ed. by Govindjee (Academic, New York, 1982), pp. 507–533.
3. F. Pliquett and M. K. Solntsev, *Thermolumineszenz biologischer objecte* (VEB Georg Thieme, Leipzig, 1978).
4. P. P. Noks, P. S. Venediktov, A. A. Kononenko, et al., Mol. Biol. **18**, 766 (1984).
5. I. Vass and Govindjee, Photosynth. Res. **48**, 117 (1996).
6. J.-M. Ducruet and I. Vass, Photosynth. Res. **101**, 195 (2009).
7. W. Arnold and N. E. Sherwood, Proc. Natl. Acad. Sci. U. S. A. **43**, 105 (1957).
8. A. K. Kukushkin, Biofizika **14**, 1124 (1968).
9. I. Vass, Photosynth. Res. **76**, 303 (2003).
10. Y. Inoue, Biochim. Biophys. Acta **634**, 309 (1981).
11. I. Vass, H. Koike, and Y. Inoue, Biochim. Biophys. Acta **810**, 302 (1985).
12. H. Koike and Y. Siderer, T. Ono, et al., Biochim. Biophys. Acta **850**, 80 (1986).
13. M. K. Solntsev, Z. P. Gribova, and V. A. Karavaev, Fiziol. Rast. **36**, 686 (1989).
14. M. K. Solntsev, Zh. Fiz. Khim. **63**, 1959 (1989).
15. V. A. Karavaev, M. K. Solntsev, T. P. Yurina, et al., Fiziol. Rast. **44**, 20 (1997).
16. T. P. Yurina, A. M. Umnov, V. A. Karavaev, et al., Fiziol. Rast. **39**, 270 (1992).
17. M. K. Solntsev, H. P. F. Ekobena, V. A. Karavaev, et al., J. Lumin. **76–77**, 349 (1998).
18. M. K. Solntsev, V. V. Frantsev, D. Yu. Shkol'nikov, et al., *Proceedings of All-Russian Conference "Unconventional and Rare Plants, Natural Compounds and Pros-*

pects for their Use" (Belgorod, 2006), Vol. 1, pp. 379–383.

19. M. K. Solntsev, V. V. Frantsev, V. A. Karavaev, et al., Collect. Sci. Pap., Ser. Crop Sci. **21**, 209 (2004).
20. E. A. Kuznetsova, V. A. Karavaev, M. K. Solntsev, et al., *Proceedings of the IV International Conference "Regulation of Growth, Development and Productivity of Plants"* (Minsk, 2005), p. 124.
21. S. A. Glazunova, V. A. Karavaev, O. I. Pokrovskii, et al., Sverkhkrit. Flyuidy. Teor. Prakt. **1**, 66 (2009).
22. M. K. Solntsev, L. E. Gunar, and V. Tashish, Izv. Ross. Akad. Nauk, Ser. Biol. **4**, 502 (1996).
23. A. M. Kuznetsov, M. K. Solntsev, V. A. Karavaev, et al., in *Modern Fungicides and Antifungal Compounds II*, Ed. by H. Lyr, P. E. Russel, H.-W. Dehne, and H. D. Sisler (Intercept, Andover, 1999), pp. 229–236.
24. S. Demeter and Govindjee, Physiol. Plant. **75**, 121 (1989).
25. O. A. Kalmatskaya, B. V. Trubitsin, I. S. Suslichenko, et al., Photosynth. Res. **146**, 123 (2020).
26. W. Arnold and J. R. Azzi, Proc. Natl. Acad. Sci. U. S. A. **61**, 29 (1968).
27. T. Ichikawa, Y. Inoue, and K. Shibata, Biochim. Biophys. Acta **408**, 228 (1975).

Translated by E. Puchkov