Influence of limiting factors on the physiologicale processes of plants of the genus *Solanum*

Dmitry Zakharov[1*], *Eugenia* Stepanova[1], *Mark* Paskhin[1], *Dina* Kazantseva[1], *Vladimir* Kosolapov[2], *Sergey* Gudkov[1] *Natalia* Semenova[1], *Alexey* Glinushkin [1,3], and *Denis* Yanykin[1]

[1] Prokhorov General Physics Institute of the Russian Academy of Sciences, 119991 Moscow, Russia;
[2] Russian Academy of Sciences, 119991 Moscow, Russia.
[3] FSBEI "State University of Enlightenment", 141014 Moscow region, Mytishchi, Russia

Abstract. The effect on transpiration in tomato on different tiers (Crisis tiers) was studied, which consisted of increasing transpiration parameters in option 1 (Standard) and option 2 (Load). The influence of regulated photosynthetic flow on the studied tomato under different conditions was studied. Changes in transpiration parameters (from $1.4*10^{-5}$ mol m^{-2} s^{-1} to 1.3*10-3 mol m^{-2} s^{-1}) are reflected in the graphs. The portable LI-COR LI-6800 system successfully measured several parameters in the same area of the leaf: photosynthesis (μmol m^{-2} s^{-1}), transpiration (mol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}). The average level of transpiration in the studied tomato on crisis tiers is different and is, in option 1 (Standard): $1.6*10^{-4}$ mol m^{-2} s^{-1}± $1*10^{-5}$ mol m^{-2} s^{-1}, in option 2 (Load): $3.1*10^{-4}$ mol m^{-2} s^{-1} ± $2*10^{-5}$ mol m^{-2} s^{-1}. The average level of transpiration of the studied tomato on the control layer, in option 1 (standard) is $2.2*10^{-4}$ mol m^{-2} s^{-1} ± $3*10^{-6}$ mol m^{-2} s^{-1}, in option 2 (Load) is $3.6*10^{-4}$ mol m^{-2} s^{-1} ± $4*10^{-6}$ mol m^{-2} s^{-1}.

1 Introduction

Agricultural research is of key importance in promoting and enriching knowledge in global science, which contributes to the development and improvement of the quality of agricultural products. Thus, many researchers have devoted a large number of their works to studying the normal growth and development of plants [1] – [5]. Under optimal conditions, the metabolic processes within individual cells, as well as between cells, organs and tissues, occur most efficiently. The search for such conditions for each plant species is an important task of scientific work.

Chlorophyll fluorescence is a promising mechanism for evaluating and analyzing the process of photosynthesis, which is continuously associated with the process of transpiration due to water exchange through stomatal channels.

There are quite a few studies that study the relationship between fluorescence and photosynthesis [6] - [7]. Such studies will certainly expand when studying external factors.

* Corresponding author: zaharov121221@mail.ru

In order to help and simplify the accumulation of data on the study of photosynthesis, an innovative device LI-COR LI-6800 was developed. This device is equipped with a visible and near-infrared spectrometer for simultaneous measurement of active and passive fluorescence, as well as software for analyzing and processing the received information.

Disruption of photosynthesis ~~is~~ a process associated with the destruction of chlorophyll and some enzymes, leading to a weakening of all synthetic processes in the plant. Lack of chlorophyll is accompanied by a decrease in the content of green pigments, i.e. loss of color intensity of leaves or their individual sections. If the change in the amount of chlorophyll in the cells is insignificant, the plant only slightly lags behind in growth and brings a reduced yield. With a strong weakening of photosynthesis, a linear (i.e. y=kx+b) result is formed (in terms of yield or quality), dwarfism of plants and, as a result, their further death often occurs. There are many factors that serve to reduce the content of chlorophyll in cells, one of these factors is plant diseases.

The object of research in this article is tomato plants. They are highly susceptible to phytopathogens, which can reduce the yield and quality of products. Among other things, tomatoes are a popular crop that is included in the list of strategic agricultural plants [On theapproval of the list of genera and types of agricultural plants, the production and cultivation of which is aimed at ensuring food security of the Russian Federation, varieties and hybrids of which are subject to inclusion in the State Register of Varieties and hybrids of Agricultural Plants approved for Use No. 3835-r], which confirms the relevance of research aimed at studying the development, course and methods of combating harmful objects affecting the phytohealth of tomatoes [8].

2 Relevance

The volume of tomato imports in the Russian Federation in 2021 amounted to 462.0 thousand tons, which is 4.4% (21.1 thousand tons) less than in 2020 (Figure 1).

Imports in 2021 were formed mainly due to supplies from Azerbaijan (30.0%), Turkey (16.1%), Uzbekistan (9.1%). Tomatoes were also imported in relatively large volumes from Turkmenistan (8.8%) and China (8.5%) [9].

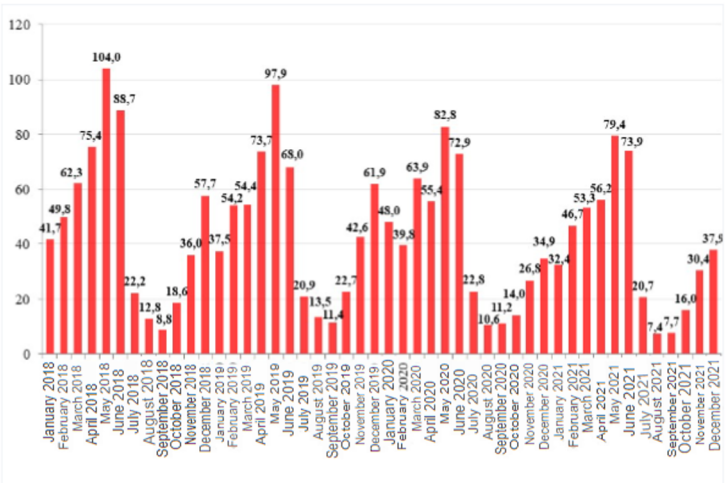

Fig. 1. Dynamics of tomato imports in Russia in 2018-2021, thousand tons.

Introduction of advanced technologies in the process of cultivation, prevention and preservation of food security in the country contributes to the reduction of tomato imports to Russia.

One of these processes is the study of the physiology of its transformation processes when changing the phytosanitary state of plants.

When studying and obtaining reference indicators of healthy and diseased plants, and with the help of their further analysis and processing of the results obtained, it is possible to develop algorithms, create a system of algorithms for determining plant diseases in the early stages of infection.

Tomatoes are affected by whiter than 60 types of pathogens [10]. Tomato crop losses due to bacterial and fungal diseases can reach 60%, so measures for disease prevention and subsequent plant protection are very important [11] - [13].

Tomato variety Daria F1. Early-ripening (85-90 days) cherry tomato hybrid with abundant brushes of mini-fruits of pink color and high sugar content. It is valued for early ripening, high yield, color, quality and taste of fruits. It is used fresh as a vegetable dessert, perfect for whole-fruit canning of a variety of assorted dishes. It is recommended for growing in protected soil in all regions of the Russian Federation. The plant is tall up to 2.5 m, weakly leaved. The brushes are powerful, complex with 35-40 fruits. The fruits are oval-cuboid, weighing 30-35g, intensely pink in color. They are characterized by high taste qualities (sugar content 7.5-8%). The potential yield is up to 12kg/m2 [14].

3 Materials and methods

The object of the study was a tomato and its leaf surface divided into 3 tiers, where: 1 - lower-located leaves, 3-upper-located leaves, 2-leaves between the first and third tiers. Hybrids were used, varieties of tomatoes for different purposes, encrypted according to the contract (Tomato variety Daria F1) for research from the customer. It was decided to use a one-factor experiment, where the pathogen was the variable factor [14 – 20].

Physiological aspects of monitoring the state and technological realization of the biological potential of tomatoes (hybrids and varieties) of different directions were carried out with the LI-6800 device (calculates the mass flow of gas intake, i.e. mmol CO of CO_2 c^{-1} and mmol of H_2O c^{-1}, into and out of the chamber, per unit time). Differences between CO_2 and H2o concentrations$_2$ и H_2in and out of the leaf cell are due to CO_2 assimilation$_2$ and transpiration processes based on the leaf surface area (mmol of CO_2 m^{-2} s^{-1} and mmol of H_2O m^{-2} s^{-1}). During photosynthetic carbon assimilation, the leaf absorbs CO_2 differently$_2$ from the air entering the cell.

Assimilation CO_2 and transpiration are related, since water vapor and carbon dioxide pass through the same stomatal openings, but these processes are highly dependent on external conditions, changing the correlation when additional factors are added. The diffusion of water vapor, like that of carbon dioxide, is different and can vary in intensity.

The importance of the quantitative and qualitative components of the pathways of photosynthesis in the environment, time and space is supported by a large preponderance of green plants, with the main$_{C3}$ pathway of photosynthesis (Calvin cycle – consisting in the carboxylation of ribulose diphosphate (C_5) to form two molecules (C_3) of 3-phosphoglycerol acid (3-FHA), part of 3-FHA molecules are reduced to 3-phosphoglycerol aldehyde (3-FHA), which later participates in the formation of$_{C6}$ photosynthetic products). The process of photosynthesis of$_{C4}$ (Hatch-Slack pathway), in contrast to$_{C3}$, got its name from the primary product of the light-independent reaction of$_{CO2}$ with phosphoenolpyruvic acid (FEPVC) with the formation of oxaloacetic acid (ACAC). In$_{C4}$ plants, photosynthesis can also be carried out

with almost closed stomata, since carbon dioxide is already stored in the tissues in the form of PIKE, closing the stomata at the hottest time of the day reduces water loss [21].

The PHYTO Scientific Circle used the LI-COR (LI-6800) device with an additional modification provided by the manufacturer: the ability to use a canister containing$_{CO2}$to change the concentrations of water and carbon dioxide between the control chamber and the leaf sampling chamber. The gas exchange parameter (net CO2 assimilation$_2$ and stomatal conductivity) was studied in a steady state (change of parameters by no more than 1% within 1 minute) at three levels, at a constant temperature [22].

According to the manufacturer's specifications for the LI-6800 instrument, the spectral response function of the red LED light source is set to 500 nm.

Closing stomata helps reduce water loss caused by transpiration in response to soil aridity. Stomatal restriction is generally considered a major factor in reducing photosynthesis under stress.

Our researchers clearly monitored the maintenance of nominal values-50 µµmol, for a stable state, which was achieved within 10 minutes. At nominal values of 500µmol and 1500 µµmol, the steady state was achieved within 20 minutes.

In the course of working with the device, a database containing a large number of parameters was obtained. In this material, two research options are considered with a change in one factor:

1. Option 1 (Standard) - Study and analysis of variable transpiration values on tomatoes that were subjected to limiting factors, but were not limited to such factors.
2. Option 2 (Load) - Analysis of the effect of photon loading on changes in transpiration parameters on the studied tomato (both in the presence of a limiting factor and in its absence).

The study of Variant 1 and Variant 2 was carried out under a constant supply of$_{CO2}$, a concentration of 400 µmol mol-1. The index of the flux density of photosynthetic photons of radiation varied according to the following variants: variant 1-0 µmol m-2 s-1, variant 2 - 1500, 1200, 900, 600, 300, 150, 50 ,0 µmol m-2 s-1.

A qualitative analysis of fetuses was carried out according to organoleptic, visual and technically controlled parameters.

4 Research results

During the experiment, we decided to consider some parameters: photosynthesis (mol $m^{-}2$ $s^{-}1$), transpiration (mol $m^{-}2$ $s^{-}1$), stomatal conductivity. (mol m^{-2} s^{-1}).

Fig. 2. Preparatory work before using the LI-COR LI-6800 equipment

The selected tomatoes were pre-prepared for observation: the studied tiers were marked; the time of day was selected; biometric data was measured, etc.

The results of measuring the transpiration parameters of option 1 (Standard) are shown in Figure 3.

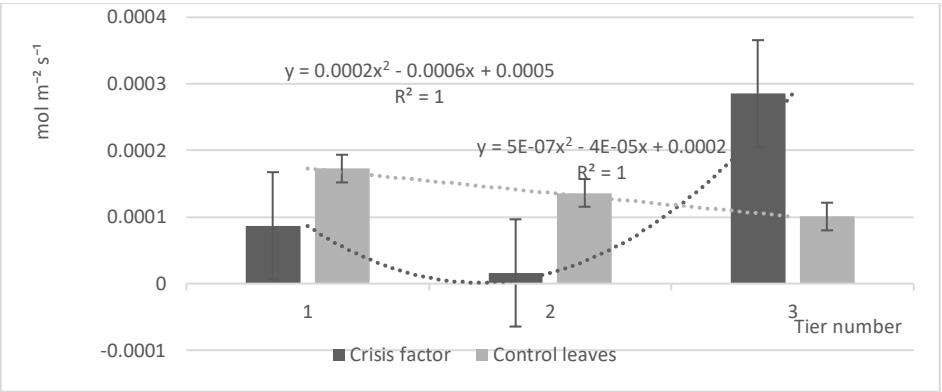

Fig. 3. Variation of the average transpiration rates of tomatoes, mol m-2 s-1.

We decided to use the considered values in the form of averaged indicators in order to simplify and facilitate further analysis and comparison of the results. To increase the accuracy of the experiment, we used the approximation and the margin of error (standard error), which are shown in the graph (Figure 3).

According to the research data, it turns out that the transpiration parameter of the object subject to the limiting factor is equal to: lower tier (1) - 8.6,6*10-5^{-5} mol m-2 s-1 ± 1*10-5^{-5} mol m-2 s-1, middle tier (2)$^{-}$2*10-5^{-5} mol m^{-}2 s -1 ± 1*10-5^{-5} mol m-2 s-1, upper tier (3) – 4*10-4^{-4} mol m^{-}2 s^{-}1 ± 1*10-5^{-5} mol m^{-}2 s^{-}1.

In the observed (control) tomato, which was not subjected to the limiting effect, the transpiration parameter is lower than in the crisis one: the upper tier (3) is 1.3*10-4^{-4} mol m-2 s-1 ± 7*10-6^{-6} mol m-2 s-1, the middle tier (2) is 2.3*10-4^{-4} mol m-2 s-1 ± 5*10-6^{-6} mol m-2 s-1,: lower tier (1) - 3*10-4^{-4} mol m-2 s-1 ± 6*10-6^{-6} mol m-2 s-1.

After analyzing the above data, we come to the conclusion that the leaves exposed to the crisis factor significantly exceed the control ones on the third tier. The remaining control tiers undergo better transpiration than those affected by the crisis factor.

For further analysis and confirmation of the pattern, we will consider the response of the transpiration of the crisis and control tiers to the wave load (Figure 4).

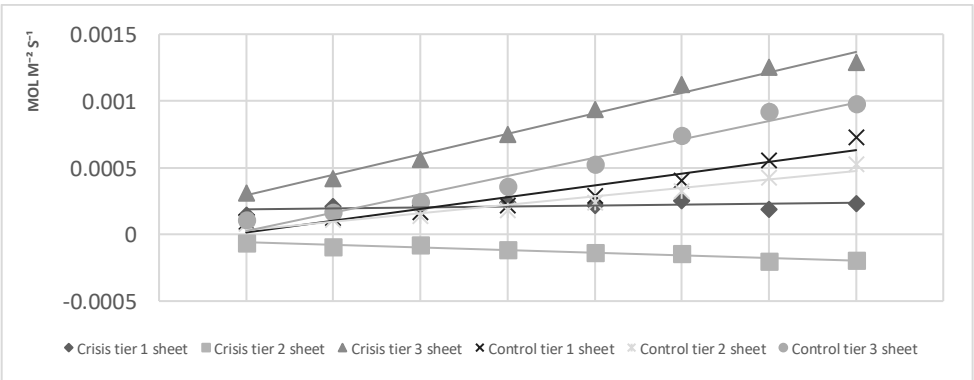

Fig. 4. Sequence of light response of encrypted tomato variety, m-2 s-1

Based on the variation of the predicted factors, it follows that the effect of photon loading on two different tomato tiers is specific. It was found that the studied leaves with a crisis factor, under the influence of a pathogen, reacted in a variety of ways to changes in wave conditions. Leaves, exposed to the limiting factor, actively change the transpiration readings, all tiers of leaves vary within each tier in different ways. This chart shows changes in the crisis series: -2*10-5^{-5} mol m-2 s-1 to 1.3*10-3^{-3} mol m-2 s-1.

The studied control tier shows a model increase in the level of transpiration, the differences in the obtained values for the control series varies : from 1 * 10-6^{-6} mol m-2 s-1 to 1*10-3^{-3} mol m-2 s-1;

At the following levels of photon vibrations: 1500, 1200, 900, 600, 300, 150, 50 ,0 µmol m-2 s-1.

According to the graph (Fig. 3, Fig. 4) and digital values (Table 1), a pattern can be traced between the two variants under study

Table 1. Research processes and averaged data on the variable scatter of transpiration indicators (average, mol m^{-2} s^{-1}).

Name	Using the crisis factor.	Without using the crisis factor (control leaves)
Option 1 (Reference)	1,6*10-4^{-4}	2,2*10-4^{-4}
Option 2 (Load)	3,1*10-4^{-4}	3,6*10-4^{-4}
Average variation deviation for leaves with a limiting factor	of 2.3*10-4^{-4}	
Average variation deviation for leaves without a limiting factor	of 2.9*10-4^{-4}	

The preparation of variable transpiration parameters led to the use of average values of the transpiration process for the selected variants, this is due to the accuracy of the resulting values. The average deviation between the variants is represented by a clear numerical value of the difference between the two factors considered: option 1 (reference) and option 2 (photon load). When calculating this parameter using standard formulas, it turned out that the control leaves have an overestimated deviation, which shows us the differences between the options.

The average value of transpiration of leaves exposed to the crisis factor of variant 1 (standard) is 1.6*10-4^{-4} mol m-2 s-1; variant 2 – 3,1*10-4^{-4} mol m-2 s-1.

Average transpiration value of the control leaves of the variant 1 – 2,2 *10-4^{-4} mol m^{-}2 s-1; options 2 – 3,6*10-4^{-4} mol m-2 s-1.

It was foundthat the difference in variants between crisis and control leaves is significant (2.3*10-6^{-6} ± 2*10-7^{-7} mol m-2 s-1 and 2.9*10-5^{-5} ± 7*10-7^{-7} mol m-2 s-1). These indicators suggest a pattern between the average deviation and the phyto-health of the studied plant.

5 Discussion

The work is carried out under an agricultural contract, which provides both material support and imposes some restrictions, and also shows that the scientific idea is interesting and ready to be supported. The prospects of the obtained research data are huge and immense.

Before starting the laboratory study, a huge amount of work was done on collecting and analyzing many literature sources, which contain little information about the LI-COR system

used, but enough information about the mechanism and principle of plant physiological processes (photosynthesis, transpiration, stomatal conduction, etc.). According to the results of which, it turned out that the optimal assimilation of CO_2 by photosynthesis at the leaf level is directly related to the loss H_2 of H_2O. The use of energy, from light photon capture during carbon assimilation and H_2O loss O_2, is coordinated by regulating stomatal conduction. Research is mainly aimed at understanding the interdependence of these regulatory mechanisms, which led to interest in the choice of methods for conducting analysis. Based on this, we chose two research methods: 1-to analyze the plant's reaction at different concentrations of CO_2; 2-to consider the effect of fluorescence at different pulses of photon radiation.

The LI-6800 system effectively measures fluorescence and gas exchange. Using the new tool and further analysis, the influence of various factors on the fluorescence intensity is checked. The results of observations explain the obvious relationship between the induced stress and the parameter under study.

It is important to note that changes in stomata and chlorophyll fluorescence will react in different time periods, but in general, this leads to a decrease in photosynthetic ability [23] - [25].

6 Conclusion

The LI-COR LI-6800 portable system successfully measured several parameters in the same leaf region: photosynthesis (mol m^{-2} s^{-1}), transpiration (mol m^{-2} s^{-1}), stomatal conductivity (mol m^{-2} s^{-1}).

The effect of regulated photosynthetic flow on the studied tomato under different conditions is studied. Changes in transpiration parameters (from 1.4 * $10\text{-}5^{-5}$ mol m-2 s-1 to 1.3*$10\text{-}3^{-3}$ mol m-2 s-1) are shown in the graphs.

Leaves exposed to the limiting factor actively change the indications of transpiration, all tiers of leaves vary..4), you can see the differences in the crisis series: -2*$10\text{-}5^{-5}$ mol m-2 s-1 to 1.3*$10\text{-}3^{-3}$ mol m-2 s-1.

The studied control tier shows a model increase in the level of transpiration,the differences in the obtained values for the control series vary: from 1 * $10\text{-}6^{-6}$ mol m-2 s-1 to 1*$10\text{-}3^{-3}$ mol m-2 s. It was found that the control leaves have an overestimated deviation, which shows us the differences between the variants.

Transpiration of the studied tomato at different tiers (Crisis tiers), which consisted in increasing the transpiration parameters in option 1 (Standard) and option 2 (Load).

The average level of transpiration in the studied tomato on crisis tiers is different and is, in option 1 (Standard): 1.6 *$10\text{-}4^{-4}$ mol m-2 s-1± 1*$10\text{-}5^{-5}$ mol m-2 s-1, in option 2 (Load): 3.1 *$10\text{-}4^{-4}$ mol m-2 s-1 ± 2*$10\text{-}5^{-5}$ mol m-2 s-1. The average level of transpiration of thestudied tomato on the control tier, in option 1 (standard) is 2.2 *$10\text{-}4^{-4}$ mol m-2 s-1 ± 3*$10\text{-}6^{-6}$ mol m-2 s-1, in option 2 (Load) is 3.6 *$10\text{-}4^{-4}$ mol m-2 s-1 ± 4 *$10\text{-}6^{-6}$ mol m-2 s-1.

It can be arguedthat the leaves exposed to the crisis factor significantly exceed the control ones on the third tier. The remaining control tiers undergo better transpiration, than ythose exposed to the crisis factor (Figure 3).

The control layerunder study, in option 2, has a graphical representation in the form of an exponent, where the largest vertex is the zero and pre-zero additional wave load (0, 50 µmol m-2 s^{-}).

The prospects of such research approaches will allow us to identify the most biologically productive systems for reproducing agricultural products with given qualitative characteristics.

References

1. Glinushkin A. P., Sokolov M. S., Toropova E. Yu. (Moscow, Agrorus Publ., 2016)
2. M. S. Sokolov, Yu. Ya. Spiridonov, E. Y. Toropova, A. P. Glinushkin A.M., Semenov (Moscow, Agrokhimiya, 2018)
3. Spiridonov, E. Y. Toropova, A. P. Glinushkin, Izvestiya Rossiiskoi akademii nauk. The biological series, 2020, **1,** 12-21
4. Kruglyak E. B., Borisova I. P., Budynkov N. I., Patent No. 2409951 C1 Russian Federation, IPC A01N 63/04. Sredstvo dlya zashchity rasteniy: no. 2009143967/10 : proklavl. 27.11.2009: publ. 27.01.2011
5. M. S. Sokolov, et.al, Research Institute of Biol. plant protection 1994
6. A.P. Glinushkin, Influence of growth conditions of Siberian cedar pine (Pinus Sibirica D u T our) on the processes of photosynthesis and transpiration (Moscow, Coniferous Boreal zones. 2022, **40(5)** pp. 361-368
7. H.G. Jones, J. Exp. Bot. 1998, **49,** 387–398
8. M. S. Sokolov, Marchenko A. I., Sanin S. S., Toropova E. Yu., [et al.]. Proceedings of the Timiryazevskaya Agricultural Academy, 2009, **1,** 13-22
9. On the import of tomatoes to Russia in 2015-2021, AB-Center URL: https://ab-centre.ru/news/ob-importe-tomatov-v-rossiyu-v-2015-2021 (accessed on 03.11.2022)
10. Detection and identification of phytopathogens, GenBit URL: https://genbitgroup.com/ru/phytopathogens
11. Yu.M. Veretennikov, et.al, Agrochemistry, 2013, **6**, 32-39
12. F. Tardieu, Simonneau, T. J. Exp. Bot. 1998**, 49,** 419–432
13. GFS-3000 Gas-Exchange System. Walz URL: https://www.walz.com/products/gas_exchange/gfs-3000/introduction.html (дата обращения: 01.10.2023).
14. N. V. Shulgin, O. A. Shulgina, *Innovations in science and practice* : A collection of articles based on the materials of the X International Scientific and practical conference. In 4 parts, Barnaul, October 02, 2018 / Responsible Editor Khalikov A.R. Volume Part **4(4) (**Barnaul, Dendra Limited Liability Company, 2018)
15. P. Kant, Y. Reinprecht, C.J. Martin, R. Islam, K.P. Pauls, 4.65 - Disease Resistance,Editor(s): Murray Moo-Young, Comprehensive Biotechnology (Third Edition), Pergamon, 2017, Pages 789-805 https://doi.org/10.1016/B978-0-12-809633-8.09244-X
16. A. O. Plotnikov, E. A. Selivanova, Yu. A. Khlopko [et al.], News of the Russian Academy of Sciences. Geographical series. 2022. **86(6),** 985-1001. https://doi.org/10.31857/S2587556622060127
17. N. R. Akmukhanova, B. K. Zayadan, A. K. Sadvakasova, Microbiology. 2022. **91,5** 533-541 https://doi.org/10.1134/s0026261722601166
18. F. F. Protopopov, I. N. Nikolaev, A. A. Alekseev [et al.], Biophysics. 2021, **66(5),** 779-785 https://doi.org/10.1134/S0006350921050195
19. D. A. Todorenko, J. Hao, O. V. Slatinskaya [et al.], Functional Plant Biology. 2021. **48(10),** 994-1004. https://doi.org/10.1071/FP21134. – EDN LEYBMR.
20. D. N. Matorin, N. P. Timofeev, D. A. Todorenko [et al.], Biophysics. 2020. **65(2),** 287-294 https://doi.org/10.1134/S0006350920020141

21. A. A. Okolelova, A. P. Glinushkin, L. L. Sviridova [et al.], Agronomy. 2022. **12(11)** 2765. https://doi.org/10.3390/agronomy12112765
22. D. Sethi, S. K. Pattanayak, S. Subudhi [et al.], Forests. 2021. 12(12) https://doi.org/10.3390/f12121657
23. V. A. Drinyaev, E. B. Kruglyak, O. I. Tikhomirova [et al.], Patent No. 2324351 C1 Russian Federation, IPC A01N 63/04. Sredstvo dlya zashchity rasteniy: no. 2006146359/15
24. Yu. Ya. Spiridonov, M. S. Sokolov, A. P. Glinushkin [et al.] (Moscow, 2019)
25. LI-6800-portable system for analyzing photosynthesis processes, LI-COR / / LABinstruments URL: https://labinstruments.ru/equipment-karbonovye-poligony-oborudovanie-dlya-analiza-gazoobmena-rasteniy/li-6800#pdf (accessed on 03.11.2022).