On the question of compared indicators of physiological development in plants of the *Solanum lycopersicum* 'Vernost' F1 under stress factor

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Abstract. On a typical Russian tomato hybrid, Solanum lycopersicum 'Vernost' F1, studies of stress factors were carried out, tracking physiological reactions to stress. Influence of Rhizopus nigricans culture was revealed on the transpiration of the studied tomato hybrid at different stress tiers, which consisted in increasing the transpiration parameters. The level of transpiration in the studied tomato plants on stress tiers is different and varied in diapason: from $1.5 *10^{-4}$ mol m-2 s-1$\pm$ $1*10^{-5}$ mol m-2 s-1 (in standard) to $3.9 *10^{-4}$ mol m-2 s-1 $\pm$ $2*10^{-5}$ mol m-2 s-1(Load). Such studies are necessary for a fundamental understanding of the possibility of biological implementation of plant hybrids.

1 Introduction

The growing world population requires new innovative solutions in agricultural technologies. To increase the yield and quality of agricultural products, a lot of fundamental and applied research is carried out, which is of great importance in world science. Many researchers have devoted their work to the study of optimal plant growth and development [1] – [5]. Such studies require a comprehensive approach and consideration of the processes occurring in plants at different levels of organization: intracellular, intercellular, organ and tissue. Optimal conditions for plant cultivation are individual for each plant species; their search is a priority task of modern agricultural science.

One of the modern methods for studying the response of plants to environmental conditions is the measurement of chlorophyll fluorescence, which allows one to assess the efficiency of the photosynthesis process, which is closely related to the process of transpiration due to water exchange through stomatal canals.

There are quite a lot of studies examining the relationship between fluorescence and photosynthesis [6-8]. To study fluorescence processes and related processes of transpiration

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and photosynthesis, the Li-COR Li-6800 instrument (LI-COR, Inc., Nebraska, USA) was developed. This equipment has a visible and near-infrared spectrometer for simultaneous measurement of active and passive fluorescence, and special software for analyzing and processing obtained data.

Measurements of gas exchange and fluorescence parameters make it possible to identify disturbances in the functioning of photosystems associated with the destruction of chlorophyll and some enzymes, as well as to estimate the content of anthocyanins and flavonols in leaves [9].

Fluorescence indicators are used on various crops to assess stress caused by various pathogens, lack of nutrients and unfavorable environmental conditions [10-12]. At the same time, a decrease in the concentration of chlorophyll is recorded, causing a loss of color intensity of the leaves. This non-invasive approach to diagnosing stress makes it possible to detect a significant decrease in chlorophyll content and detect stress at an earlier time to prevent yield losses.

The object of our research was tomato plants. They are susceptible to diseases of various etiologies, especially of fungal origin, which significantly reduces the yield and quality of products. Fluorescence measuring of tomato plants is used for various purposes: determining the maximum concentration of agrochemicals [13], determining the effectiveness of the use of biotic treatments [14], including under salt stress [15], identifying the response of plants to biotic stress caused by fungal pathogens [16], as well as the effectiveness of using various substrates [17] and diagnosing plant responses to others factors that can cause stressful conditions.

By obtaining fluorescence indicators and gas exchange parameters of reference plants and plants under stress conditions, it is possible to develop algorithms for determining stress state in the early stage of development.

2 Materials and methods

The object of the study was a *Solanum lycopersicum* 'Vernost' F1 (Siberian Garden, Russia) which is a representative of a series of modern hybrids of a new generation, allowing to obtain super-yields in open ground conditions and film greenhouses. 'Vernost' F1 is early ripening (from germination to the beginning of fruit ripening 100-103 days). The plant is determinate, 120-130 cm high. Fruiting lasts until late autumn. The fruits are round, dense, without a green spot at the stalk, bright red in color, weighing 200-210 g. The hybrid has complex resistance to major diseases. Productivity 16-18 kg per 1 sq. m. The plants leaf surface was divided into 3 tiers, where: 1 - lower-located leaves, 3-upper-located leaves, 2-leaves between the first and third tiers.

Monitoring of the state and technological implementation of the biological potential of *Solanum lycopersicum* 'Vernost' F1 was carried out using a Li-COR Li-6800 device (LI-COR, Inc., Nebraska, USA). The mass flow of gas, i.e., μmol CO_2 s^{-1} and mmol $HH2O$ s^{-1}, entering and leaving the chamber per unit time was calculated. Differences in the concentrations of CO_2 and H_2O inside and outside the leaf cell are due to the processes of CO_2 assimilation and transpiration depending on the leaf surface area (μmol CO_2 m^{-2}s^{-1} and mmol H_2O m^{-2}s^{-1}).

The processes of CO_2 assimilation and transpiration are related, since water vapor and carbon dioxide pass through the same stomatal openings. These processes are highly dependent on environmental factors, so the diffusion of water vapor, like carbon dioxide, varies in intensity depending on plant water and environmental conditions.

We used the Li-COR Li-6800 with an additional modification provided by the manufacturer: the ability to use two CO_2 cylinders to vary the concentration of water and carbon dioxide between the control chamber and the leaf sampling chamber. The gas

exchange parameter (net CO_2 assimilation and stomatal conductance) was studied in stationary mode (parameter changes by no more than 1% within 1 minute) at three levels, at a constant temperature.

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

Stomatal closure helps reduce water loss caused by transpiration in response to soil aridity. Stomatal restriction is generally considered to be a major factor in the reduction of photosynthesis under stress conditions.

Our researchers carefully monitored the maintenance of a nominal value of 50 μmol, a stable state that was achieved within 10 minutes. At nominal values of 500 μmol and 1500 μmol, steady state was reached within 20 minutes.

The masses of data obtained from the device were used to form a database containing a large number of parameters.

We set up two research options with a change in one factor:

1. Variant (Standard) - Study and analysis of variable transpiration values on tomatoes exposed to the limiting factor, and not limited to it.

2. Variant 2 (Load) - Analysis of the influence of photon load on changes in transpiration parameters on the tomato under study (both in the presence of a limiting factor and in its absence).

The study of Option 1 and Option 2 was carried out with a constant supply of CO_2, concentration 400 μmol mol^{-1}. The flux density of photosynthetic photons of radiation varied according to the following options: option 1-0 μmol m^{-2} s^{-1}, option 2 - 1500, 1200, 900, 600, 300, 150, 50.0 μmol m^{-2} s^{-1}.

We used the pathogen inoculation of *Rhizopus nigricans* as a variable factor.

Qualitative analysis of the fruits was carried out according to organoleptic, visual and technically controlled indicators. All experiments were carried out in triplicate, followed by statistical processing and plotting were performed in MS Excel 2010. One-way analysis of variance (ANOVA) with a significant difference of $P < 0.05$ was used to determine significant differences.

3 Research results

During the experiment (Figure 1), 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. 1. LI-COR LI-6800 data obtaining from *Solanum lycopersicum* 'Vernost' F1.

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

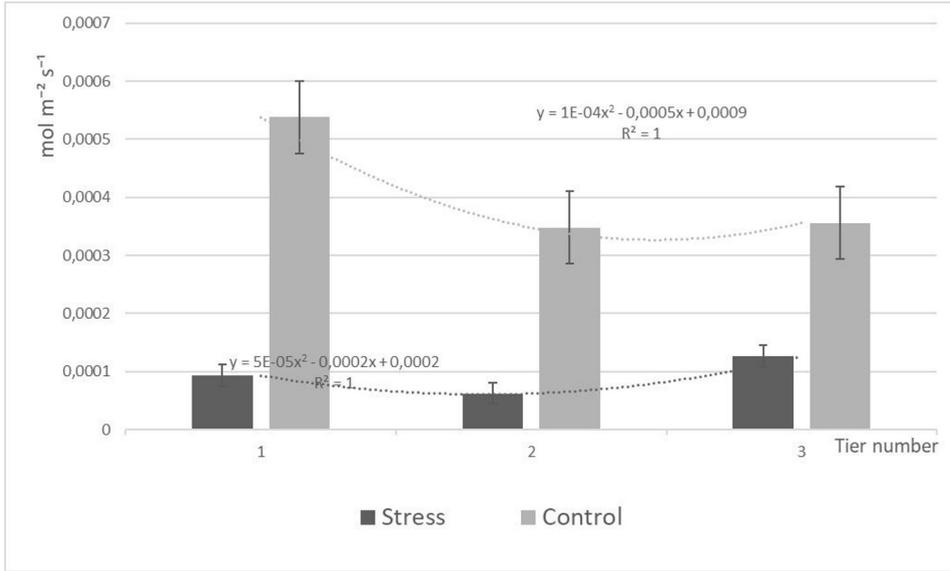The results of measuring the transpiration parameters of variant 1 (Standard) are shown in Figure 2.

Fig. 3. Transpiration of *Solanum lycopersicum* 'Vernost' F1, 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, the approximation and error limit were used, which are reflected in the graph (Figure 3).

According to research data, it turns out that the transpiration parameter of an object subject to the limiting factor is: lower tier (1) – $1.5*10^{-4}$ mol m^{-2} s^{-1} ± $2*10^{-5}$ mol m^{-2} s^{-1}, middle tier (2) - $1.5*10^{-4}$ mol m^{-2} s^{-1} ± $1*10^{-5}$ mol m^{-2} s^{-1}, upper tier (3) – $3.9*10^{-4}$ mol m^{-2} s^{-1} ± $1*10^{-5}$ mol m^{-2} s^{-1}.

When, as in the observed tomato, which was not subjected to the limiting effect, the transpiration parameter is lower: the upper tier (3) is $3.4*10\text{-}4^{-4}$ mol m^{-2} s^{-1} ± $5*10^{-6}$ mol m^{-2} s^{-1}, the middle tier (2) is $2.9*10^{-5}$ mol m^{-2} s^{-1} ± $5*10^{-6}$ mol m^{-2} s^{-1}: lower tier (1) – $3.2*10^{-5}$ mol m^{-2} s^{-1} ± $7*10^{-6}$ mol m^{-2} s^{-1}.

After analyzing the above data, we come to the conclusion that the leaves exposed to the stress factor on the first and second tiers are much lower than the indicators on the same tiers (except for the upper tier), but without limiting effects.

Comparing the indicators of the upper tier (3), with and without the stress component, we can conclude that the differences are not significant.

The visualized information (Figure 3) tells us that между здоровыми и больными there is a difference in the strength of transpiration between healthy and diseased leaves. For subsequent discussions, the average transpiration values of the healthy and diseased tiers will be used.

So, the obtained параметрыtranspiration parameters will serve as a starting point for comparing the results.

For further analysis and confirmation of the pattern, we will consider the response of transpiration of the sick and healthy tiers to light flashes (Fig. 3).
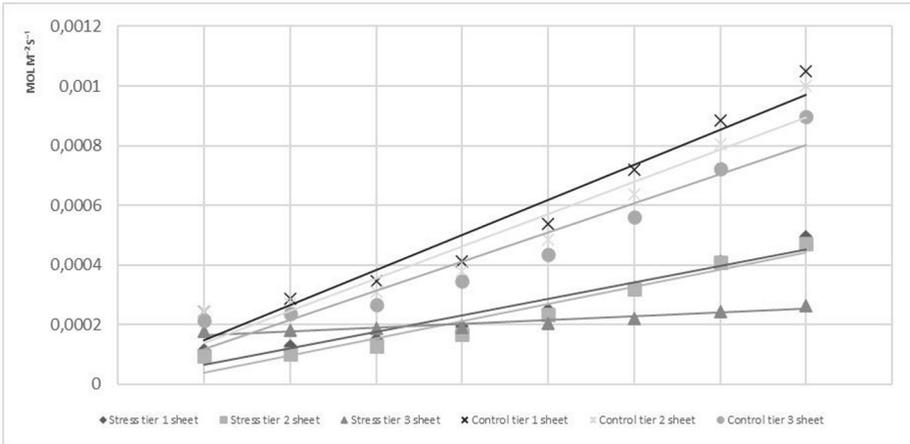
Fig. 4. Sequence of light response of Solanum lycopersicum 'Vernost' F1, m-2 s-1

Based on the variation of the predicted factors, it follows that the effect of photon loading on two different tomato tiers is specific. We found that the studied leaves with a stress factor, under the influence of a pathogen, модельнореacted in a model way to changes in wave conditions. Leaves exposed to the *Rhizopus nigricans* inoculation as limiting factor (or free from it) actively change and increase their transpiration parameters.

The studied tiers show a model increase in the level of transpiration and have a graphical representation in the form of an exponential, where the largest peak is zero and pre-zero additional wave load (0.50 μmol m^{-2} s^{-1}).

Option 2, with a photon load, showed the following spread of the obtained values for the control series: from $2.5*10^{-4}$ mol m^{-2} s^{-1} to $6.9*10^{-4}$ mol m^{-2} s^{-1}; stress series: $2.1*10^{-4}$ mol m^{-2} s^{-1} to $4*10^{-4}$ 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 spread of transpiration indicators (average, mol m^{-2} s^{-1}).

Name	Using the stress factor (inoculated leaves).	Without using the stress factor (control leaves)
Option 1	$3.2*10^{-4}$	$1.5*10^{-4}$
Option 2	$3.1*10^{-4}$	$3.9*10^{-4}$
Average deviation of diseased leaves	$1.7*10^{-4}$	
Average deviation of healthy leaves	$3*10^{-5}$	

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 stressed leaves have an underestimated deviation.

The average value of transpiration of leaves exposed to the stress factor of variant 1 (standard) is $1.5*10^{-4}$ mol m^{-2} s^{-1}; variant 2 – $3,9*10^{-4}$ mol m^{-2} s^{-1}.

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

According to the studied information, it turned out that the difference in the selected variants in the stressed and control leaves is significant ($1.7*10^{-4} \pm 2*10^{-5}$ mol m^{-2} s^{-1} and $3*10^{-5} \pm 7*10^{-7}$ mol m^{-2} s^{-1}). These indicators suggest a pattern between the average deviation and the phyto-health of the studied plant.

4 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_2O. The use of energy, from light photon capture during carbon assimilation and H_2O loss 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 [18-21].

5 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 $3.2 * 10^{-5}$ mol m^{-2} s^{-1} to $3.9*10^{-4}$ mol m^{-2} s^{-1}) are shown in the graphs.

Influence of *Rhizopus nigricans* culture was revealed on the transpiration of the studied tomato hybrid at different stress 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 stress tiers is different and amounts, in option 1 (Standard): $1.5 *10^{-4}$ mol m^{-2} s$^{-1} \pm 1*10^{-5}$ mol m^{-2} s^{-1}, in option 2 (Load): $3.9 *10^{-4}$ mol m^{-2} s$^{-1} \pm 2*10^{-5}$ mol m^{-2} s^{-1}. The average level of transpiration of the studied tomato on the control tier, in option 1 (standard) is $3.22 *10^{-4}$ mol m^{-2} s$^{-1} \pm 3*10^{-6}$ mol m^{-2} s^{-1}, in option 2 (Load) is $3.1 *10^{-4}$ mol m^{-2} s$^{-1} \pm 4 *10^{-6}$ mol m^{-2} s^{-1}.

For stressed leaves under the influence of photon loading, in option 2, the transpiration efficiency is better than in option 1. When using a wave load on the studied stress layer, the

overall level of transpiration increases, compared to the control leaves. The stress stage under 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^{-1}).

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

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