PHYSIOLOGICAL RESPONSE OF SOME TOMATO GENOTYPES (LYCOPERSICON ESCULENTUM L.) TO HIGH TEMPERATURE STRESS ФИЗИОЛОГИЧЕН ОТГОВОР НА НЯКОИ ГЕНОТИПИ ПРИ ДОМАТИ (LYCOPERSICON ESCULENTUM L.) КЪМ ВИСОКОТЕМПЕРАТУРЕН СТРЕС

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ABSTRACT

Cultivated plants are often exposed to different types of environmental stress, which limits their development and productivity. High temperature stress induces considerable changes in the biochemistry and physiology of plants.

The aim of the present investigation was to evaluate the physiological response of some tomato genotypes to a high temperature stress. Experiments were carried out during the stress period and after its elimination. Valuation of the tolerance of plants was carried out by means of a physiological test. It was observed that the high temperature stress has an inhibitory effect on the physiological state of tomato plants. The degree of its inhibited action was individual, depending on the genotype.

KEY WORDS: Lycopersicon esculentum L., high temperature stress, physiological test, leaf gas-exchange, photosynthetic pigments

РЕЗЮМЕ

Културните растения често са подложени на различни видове стрес от околната среда, което ограничава тяхното развитие и продуктивност. Високотемпературният стрес предизвиква значителни изменения в биохимията и физиологията на растенията.

Целта на настоящата разработка е да даде оценка на физиологичния отговор на някои доматени генотипове на високотемпературния стрес. Опитите бяха направени по време на действието на стреса, както и след неговото отстраняване. Оценката на издръжливостта на растенията беше извършена с помощта на физиологичен тест. Беше установено, че високотемпературния стрес оказва инхибиращо въздействие върху физиологичното състояние на доматените растения. Степента на инхибирането беше индивидуална, в зависимост от генотипа.

КЛЮЧОВИ ДУМИ: Lycopersicon esculentum L., високотемпературен стрес, физиологичен тест, листен газообмен, фотосинтетични пигменти



INTRODUCTION

High temperatures are one of the environmental factors which cause modifications in the functional state of the plant organisms. From an agronomic point of view and practical experience, high temperatures are not only a meteorological event, but also a stress factor strongly influencing plant productivity. Increase in temperatures to over 40 °C, which is common for our climatic conditions, puts to the test a large number of species and plant cultivars. They are affected in different ways but the most common disturbances are reduced mainly to colloid and chemical [8, 9, 11], physiological and biological [2, 3, 4, 15, 16] and morphological [1, 5, 9] modifications and have a negative effect upon the normal functioning of the plant organism as a whole [6, 12].

The main ways of increasing the plant tolerance to stress factors, and to high temperatures in particular, are related to the application of appropriate agro-technical events (for example fertilization, treatment with bio-active substances) and to the selection of tolerant cultivars. The achievement of the desired effect by means of genetics and selection is a slow and time-consuming process. This is why it can be considered that the application of screening methods for determination of the plant tolerance is a fast and reliable method for the achievement of the desired results in the early stages of their ontogenesis. Part of these screening methods may be related to a number of physiological parameters stated as stress indicators – content of photosynthetic pigments, leaf gas exchange, fluorescence intensity, electrolytic leakage,

lipid peroxidation, etc. [17].

The purpose of the present research work was to give a fast and efficient assessment of the tolerance to high temperatures of some tomato genotypes with the help of an appropriate physiological test. For these purposes experiments were carried out in which the reaction of the genotypes in the ontogenesis was followed simultaneously with the stress development and after its elimination. At the same time research was carried out under field conditions determining the efficiency of the selected physiological indices for this type of analysis.

MATERIAL AND METHODS

The objective imposed the conduct of a complex research in which vegetation and field experiments have been used.

The vegetation experiments incorporated a preliminary developed system of physiological indices which gave information about the deviations of the main physiological processes from the standard and the time necessary for achievement of the new stationary condition. The analysis of the index system determined which of them give the fastest and most reliable indication of the stress. The separate genotypes were characterized with respect to their tolerance to the high temperature stress. The final assessment of the tolerance of the tested genotypes was made on the basis of field experiments.

The experiments were carried out on determinant largefruited tomatoes – intended for fresh consumption and

Table 1. Environmental conditions during the experimental period (glass-steel greenhouse); T₀ - 10 o'clock - day 1; T₁ - 12 o'clock - day 1; T₂ - 14 o'clock - day 1; T₃ - 16 o'clock - day 1;

T₄ - 18 o'clock - day 1; T₅ - 10 o'clock - day 2;

Таблица 1. Условия на средата през опитния период (стоманено-стъклена оранжерия); T₀ - 10 часа - 1-ви ден; T₁ - 12 часа - 1-ви ден; T₂ - 14 часа - 1-ви ден; T₃ - 16 часа - 1-ви ден;

T ₄ - 1	8 часа - 1-	ви ден; Т ₅	- 10 часа -	2-ри ден;		
Indices	To	T_1	T_2	T ₃	T_4	T ₅
Light intensity [µmol m ⁻² s ⁻	1100	1400	1700	1600	1350	950
1]						
Air temperature [°C]	32.9	36.2	43.2	39.5	34.0	32.0
Leaf temperature [°C]	31.1	35.6	39.3	37.5	32.9	30.0
Relative air humidity [%]	70	65	60	60	65	70

 Table 2. Meteorological data for the tested period;

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паолица 2. Метеорологични данни за отчетния период,			
Indices	Maximum air temperatures [°C]	Relative air humidity	
Months		[%]	
June	32,0 - 34,6	65 - 78	
July	33,7 - 34,7	58 - 66	
August	33,0 - 34,0	60 - 63	

production of juices and concentrates (Trapezitsa, Milyana, Stella, Solaris, Marty, Topaz). The enlisted cultivars are intended for middle early production. The cultivars Trapezitsa and Milyana are distinguished for their good adaptability which makes them preferred by many producers. They are adopted as standards for the examined cultivars.

A. Vegetation experiment

Initially the plants were grown in a polyethylene greenhouse at optimal conditions. In the phase of third – fourth developed leaf the tomato plants were removed to a glass and steel greenhouse and were exposed to a high temperature stress.

During the experimental period the daily course of the photosynthesis and transpiration was observed. The analyses were made in two-hour interval, in the hours T_0 , T_1 , T_2 , T_3 , T_4 μ T_5 , taking into consideration that:

 $T_0 - 10$ o'clock - day 1; $T_1 - 12$ o'clock - day 1; $T_2 - 14$ o'clock - day 1;

 $T_3 - 16$ o'clock - day 1; $T_4 - 18$ o'clock - day 1; $T_5 - 10$ o'clock - day 2;

The parameters of the leaf gas exchange – net photosynthesis (P_N), transpiration intensity (E) and stomatal conductance (g_s), were measured with a portable photosynthetic system LCA-4 (ADC, Hoddesdon, England). The first leaves over the first bunch were used for the analyses. The measurements were carried out with intact plants.

The content of the plastid pigments was defined in two

points:

 $T_0 - 10 \text{ o'clock} - \text{day 1}; \quad T_6 - 10 \text{ o'clock} - \text{day 3};$

The pigments were extracted with 85% acetone solution. The optical density of the extracts was measured spectrophotometrically, after centrifuge of the extracts at 3000 g for 5 min [13] and the pigment quantity - calculated under Lichtenthaler [10]. Leaves of identical physiological age were used.

During the experiment observations of the meteorological status were made (Table 1).

Б. Field experiment

The experiment was directed towards: 1. Studying of the rate of reaction of tomato genotypes to a high temperature stress; 2. Selection of the genotypes with different stress resistance.

The field experiment was carried out at the base of "Maritsa" Institute of vegetable crops – Plovdiv – experimental area of 1 000 sq.m.

The experiments were carried out on the tomato cultivars indicated above.

They were conducted under a standard method, in accordance with the agro-technical equipment adopted for the cultivars. Each tested cultivar was bedded in 4 repetitions, including 24 tested plants in one repetition and the tested area was 4.6m². During the tested period regular phenological observations of the plant development were made.

Detailed meteorological data from the station of the experimental field were used (Table 2). Typical for the

Table 3. Content of photosynthetic pigments (mg g^{-1} fresh weight) in tomato plants exposed to a high
temperature stress during vegetation experiments; $T_0 - 10$ o'clock – day 1; $T_6 - 10$ o'clock – day 3;
Габлица 3. Съдържание на пластидни пигменти (mg g ⁻¹ св. тегло) в растения от домати подложени на
високотемпературен стрес в условия на вегетационни опити;

	$T_0 - 10$ часа - 1-ви	и ден; Т ₆ – 10 часа –	3-ти ден;
Index	Chlorophyll a	Chlorophyll b	Chlorophyll <i>a/b</i>
Cultivar		T ₀	
Trapezitsa	1,85±0,06	0,88±0,04	2,10±0,04
Milyana	1,94±0,02	0,81±0,02	2,40±0,03
Stella	$1,72\pm0,08$	0,80±0,01	2,15±0,04
Solaris	1,34±0,04	0,52±0,01	2,60±0,06
Marty	1,18±0,06	0,62±0,02	1,90±0,02
Topaz	1,24±0,05	0,62±0,04	2,00±0,02
		T_6	
Trapezitsa	1,68±0,01	0,80±0,02	2,10±0,01
Milyana	$1,70\pm 0,02$	0,74±0,01	2,30±0,04
Stella	1,53±0,02*	0,78±0,04	1,96±0,01
Solaris	1,19±0,04*	0,57±0,01	2,01±0,02*
Marty	0,80±0,05**	0,48±0,03*	1,68±0,02*
Topaz	0,87±0,04**	0,53±0,05*	1,65±0,05*

Index	Early ripeness	Total yield
Cultivars		
Stella	1425±22,5	4630±32,0
Solaris	1328±13,2	4550±35,5
Marty	1435±18,3	4880±45,0
Trapezitsa	1420±21,0	4630±46,0
Topaz	1350±14,5	4750±32,5
Milyana	$1465\pm 26,0$	4510±24,5

Table 4. Early ripeness and total yield [kg da⁻¹] in tomato plants cultivated under field conditions; **Таблица 4.** Ранозрелост и общ добив [kg da⁻¹] при растения от домати отглеждани при полски условия;



Fig. 1. Net photosynthetic rate P_N [µmol CO₂ m⁻² s⁻¹] in tomato plants exposed to a high-temperature stress under conditions of vegetation experiments; $T_0 - 10$ o'clock; $T_1 - 12$ o'clock; $T_2 - 14$ o'clock; $T_3 - 16$ o'clock; $T_4 - 18$ o'clock; $T_5 - 10$ o'clock - day 2;

Фиг. 1. Скорост на фотосинтезата P_N [µmol $CO_2 \text{ m}^{-2} \text{ s}^{-1}$] в растения от домати подложени на високотемпературен стрес в условия на вегетационни опити; $T_0 - 10$ часа; $T_1 - 12$ часа; $T_2 - 14$ часа; $T_3 - 16$ часа; $T_4 - 18$ часа; $T_5 - 10$ часа - 2-ри ден;

trial period was that the temperature values were higher than the normal ones for the period. For the period June 1^{st} – July 20th the average 10-days temperatures were from 1.0 to 2.9°C higher than the climatic values.

The reaction of the genotypes was followed simultaneously with the development of a natural high temperature stress and in the process of its elimination. During the trial period the indices stated during the vegetation experiment were tested.

Two independent experiments were conducted (2005-2006). The results showed similar tendencies. Data from one representative experiment are given in this work.

The results were statistically processed. The authenticity

of the differences was determined according to the criterion t of Student.

RESULTS AND DISCUSSION

Temperature is an important environmental factor, determining the intensity of the process of photosynthesis. It is well known that the speed of each unit of this integral process is directly influenced by the temperature (with the exception of the early photochemical reactions which are relatively more tolerant to high temperatures).

Photosynthesis in cultural crops from the temperate climatic area is intense within the temperature interval



Fig. 2. Transpiration rate E [mmol m⁻² s⁻¹] in tomato plants exposed to a high temperature stress under conditions of vegetation experiments; $T_0 - 10$ o'clock; $T_1 - 12$ o'clock; $T_2 - 14$ o'clock; $T_3 - 16$ o'clock; $T_4 - 18$ o'clock; $T_5 - 10$ o'clock - day 2;

Фиг. 2. Интензивност на транспирацията Е [mmol m⁻² s⁻¹] в растения от домати подложени на високотемпературен стрес в условия на вегетационни опити; $T_0 - 10$ часа; $T_1 - 12$ часа; $T_2 - 14$ часа; $T_3 - 16$ часа; $T_4 - 18$ часа; $T_5 - 10$ часа - 2-ри ден;



Fig. 3. Stomatal conductance $g_s [mol m^{-2} s^{-1}]$ in tomato plants exposed to a high temperature stress during vegetation experiments; $T_0 - 10$ o'clock; $T_1 - 12$ o'clock; $T_2 - 14$ o'clock; $T_3 - 16$ o'clock; $T_4 - 18$ o'clock; $T_5 - 10$ o'clock - day 2;

Фиг. 3. Устична проводимост g_s [mol m⁻² s⁻¹] в растения от домати подложени на високотемпературен стрес в условия на вегетационни опити; $T_0 - 10$ часа; $T_1 - 12$ часа; $T_2 - 14$ часа; $T_3 - 16$ часа; $T_4 - 18$ часа; $T_5 - 10$ часа – 2-ри ден;

from 20 to 25 °C, and further increase to 40 °C, causes inhibition of the process. The stressful high temperatures cause disturbances in the photosynthetic apparatus which leads to reduction of the photosynthetic activity, damage and subsequent perish of the plants [9].

The data represented in Figures 1 and 2 shows that during the impact of extremely high temperatures on the plants of the tested tomato genotypes the daily course of the photosynthesis and transpiration becomes complex. The drastic inhibition of the parameters of the leaf gas exchange – the intensity of the photosynthesis and the transpiration in the midday and afternoon hours is related to a sharp decrease of the stomatal conductance (Figure 3). The reduced values of g_s show that to a large extent the inhibition of the photosynthesis is related with stomatal conductance limitation.

There are also cultivar differences with reference to the dynamics in the change of the parameters of the leaf gas exchange of the plants.

Under the influence of high temperatures the cultivars Topaz and Marty are characterized by significant deviations in the intensity of the photosynthesis. After the elimination of the stress the recovery of those cultivars is hard. The inhibition of the photosynthesis in the cultivars Stella and Solaris is considerably lower. After the elimination of the stress the plants recover completely. The cultivars Trapezitsa and Milyana adopted as standards take intermediate position with respect to this index. The dynamics of the changes in the intensity of the transpiration has a similar nature.

The reduced intensity of the photosynthesis (observed during the midday and afternoon hours) accompanied by low stomatal conductance and reduced intensity of the transpiration gives a reason for the assumption that the reduced intensity of the integral photosynthetic process is a result of deteriorated water status of the plants caused by the high temperature stress.

The photosynthetic pigments are one of the internal factors which can limit the photosynthetic activity to a large extent. It is proven that the reduction of the pigment concentration is an indicator of stress in cases as water and temperature stress, insufficiency or excess of mineral elements, etc. [7].

The data from Table 3 show that high temperatures cause disturbances in the photosynthetic apparatus. As a result of their influence the content of chlorophyll a in the leaves of the studied plants is reduced by 10-32%.

Cultivar differences are available. The most clearly indicated changes in the content of chlorophyll a are observed in the cultivars Marty and Topaz. As for chlorophyll b, the changes are less visible but follow the same tendency. An exception is Solaris cultivar where increased pigment content is observed.

According to some authors leaf aging in grain crops is a primary response to high temperature [2]. On the other





Фиг. 4. Брой цветове и плодове върху първа цветна китка при растения от домати подложени на високотемпературен стрес при условия на вегетационни опити;



Fig. 5. Net photosynthetic rate P_N [µmol $CO_2 \text{ m}^{-2} \text{ s}^{-1}$] in tomato plants cultivated under field conditions; $T_0 - 10$ o'clock; $T_1 - 12$ o'clock; $T_2 - 14$ o'clock; $T_3 - 16$ o'clock; $T_4 - 18$ o'clock; $T_5 - 10$ o'clock - day 2; Фиг. 5. Скорост на фотосинтезата P_N [µmol $CO_2 \text{ m}^{-2} \text{ s}^{-1}$] в растения от домати отглеждани при полски условия; $T_0 - 10$ часа; $T_1 - 12$ часа; $T_2 - 14$ часа; $T_3 - 16$ часа; $T_4 - 18$ часа; $T_5 - 10$ часа - 2-ри ден;



Fig. 6. Transpiration rate E [mmol m⁻² s⁻¹] in tomato plants cultivated under field conditions; $T_0 - 10$ o'clock; $T_1 - 12$ o'clock; $T_2 - 14$ o'clock; $T_3 - 16$ o'clock; $T_4 - 18$ o'clock; $T_5 - 10$ o'clock - day 2; Фиг. 6. Интензивност на транспирацията E [mmol m⁻² s⁻¹] в растения от домати отглеждани при полски условия; $T_0 - 10$ часа; $T_1 - 12$ часа; $T_2 - 14$ часа; $T_3 - 16$ часа; $T_4 - 18$ часа; $T_5 - 10$ часа - 2-ри ден;



Fig. 7. Stomatal conductance $g_s [mol m^{-2} s^{-1}]$ in tomato plants cultivated under field conditions; $T_0 - 10$ o'clock; $T_1 - 12$ o'clock; $T_2 - 14$ o'clock; $T_3 - 16$ o'clock; $T_4 - 18$ o'clock; $T_5 - 10$ o'clock - day 2; Фиг. 7. Устична проводимост $g_s [mol m^{-2} s^{-1}]$ в растения от домати отглеждани при полски условия; $T_0 - 10$ часа; $T_1 - 12$ часа; $T_2 - 14$ часа; $T_3 - 16$ часа; $T_4 - 18$ часа; $T_5 - 10$ часа - 2-ри ден;



Fig. 8. Number of blossoms and fruits on the first bunch in tomato plants cultivated under field conditions; Фиг. 8. Брой цветове и плодове върху първа цветна китка при растения от домати отглеждани при полски условия;

part, the reduction of chlorophyll content with the increase in temperature can be viewed as an adaptive reaction related to increased resistance to dryness: absorption of less viable energy and additional heating of the leaves when the plants suffer from insufficient water quantity and their stomatal conductivity is restricted.

The reduced values of chlorophyll a and b as well as the chlorophyl a/b ratio can be related to disturbances in the structure of the chloroplasts ascertained by other authors [14].

The changes in the pigment ratio, determined in Marty, Topaz, Stella and Solaris cultivars, are indicators of stress.

The number of formed fruits is an important component of the yield. The results show (Figure 4) that the number of fruits formed upon the first bunch is the highest in Trapezitsa cultivar, followed by Milyana cultivar. The cultivars Marty and Topaz are characterized by the lowest values with respect to this index. The increased number of fruits is determined by the larger number of blossoms and the increased pollen viability. The study of the pollen shows that the plants from all tested cultivars react negatively to high temperatures. The number of plants and blossoms with non-viable pollen increases.

A small percentage of anther seeds grow in the viable blossoms, the anther tubes very often do not reach the necessary length in order to reach pollination. Extremely sensitive to the high temperatures are the cultivars Marty and Topaz (non-published data).

The research on the influence of the temperature stress upon the pollen viability shows that on the basis of a nonidentical reaction of the male gametophyte, the studied genotypes are possible to be differentiated and to be selected those ones tolerant to this abiotic factor. This will be subject of another research of ours.

Studies determining the efficiency of the selected physiological indices for this type of analysis were carried out under field conditions.

The data represented in Figures 5 and 6 show that the changes in the parameters of the leaf gas exchange in plants cultivated under field conditions in the midday hours follow the tendency indicated during the vegetation experiments.

The observed inhibition of the intensity of the photosynthesis and transpiration is related to reduction of the stomatal conductance (Figure 7). In the afternoon hours however, the processes of photosynthesis and transpiration recover in all tested cultivars and on the following day they have the original indices. Under field conditions inhibition of the leaf gas exchange is lower during the whole studied period.

The results show (Figure 8) that the number of fruits formed upon the first bunch is the highest in Marty cultivar, followed by Stella and Topaz cultivars. The cultivars Milyana and Trapezitsa are characterized by the lowest values with respect to this index.

The studies of the pollen showed that under field conditions 40% of the plants showed good pollen viability (60-100%). The highest values of this index were detected in Stella and Solaris cultivars (non-published data).

The results represented in Table 4 show that the highest yield was indicated in Marty cultivar, followed by Topaz cultivar. The lowest yield had the cultivars Milyana and Solaris. Stella and Trapezitsa occupy a medium position with respect to this index.

At this stage of the research comparison of the results obtained from plants exposed to a high temperature stress with those cultivated under field conditions gives a reason for the assumption that Marty and Topaz are more sensitive to extremely high temperatures compared to the rest of the cultivars.

CONCLUSION

On the basis of the presented data we can outline the following physiological indices as reliable indicators of the high temperature stress: leaf gas exchange parameters – intensity of photosynthesis, intensity of transpiration, stomatal conductance, content of chlorophyll a and chlorophyll b, co-relation between chlorophyll a and chlorophyll b.

The comparison of the results obtained in plants exposed to a high temperature stress with those cultivated under field conditions gives a reason for the following assumption: Marty and Topaz cultivars are more sensitive to extremely high temperatures compared with the rest of the tested cultivars.

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