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РЕАКЦИЯ НА ФОТОСИНТЕТИЧНИЯ АПАРАТ И МЪЖКИЯ ГАМЕТОФИТ ПРИ ПИПЕР (*CAPSICUM ANNUUM* L.) КЪМ РАЗЛИЧНИ ВИСОКОТЕМПЕРАТУРНИ РЕЖИМИ
RESPONSE OF THE PHOTOSYNTHETIC APPARATUS AND MALE GAMETOPHYTE OF PEPPER PLANTS (*CAPSICUM ANNUUM* L.) TO VARIOUS HIGH TEMPERATURE REGIMES

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Резюме

Изследвани са промените в параметрите на хлорофилната флуоресценция и в мъжкия гаметофит под влияние на висока температура (ВТ) с цел да се определи подходящ температурен режим за тестиране на генотипи от *Capsicum annuum* L. Растенията (сорт „Бял Калинков") са отгледани в оранжерия в 5 L пластмасови съдове с торфено-почвен субстрат. През фаза бутонизация–цъфтеж целите растения са третирани с ВТ (35, 40 и 45°C и продължителност 1, 2, 3 и 4 h). Беше установено, че пиперовите растения се повлияват в различна степен от приложените температурни режими. Можем да обобщим, че: стойностите 35°C и 45°C не са подходящи за тестиране на линии и сортове пипер към ВТ; мъжкият гаметофит е по-чувствителен към ВТ в сравнение с фотосинтетичния апарат; установен е полулетален ефект върху мъжкия гаметофит на растения от моделния сорт „Бял Калинков" при температура 40°C с продължителност 2 и 3 h; промените в параметрите на хлорофилната флуоресценция са по-добре изразени при температурните режими с по-високи стойности (40°C и 45°C за 2 и 3 h); подходящ температурен режим за тестиране на термотолерантността на пипер включва стойност 40°C и продължителност 2 или 3 h.

Abstract

The changes in chlorophyll fluorescence parameters and in male gametophyte caused by high temperature (HT) were studied to determine a suitable regime to test the heat tolerance of *Capsicum annuum* genotypes. The plants (cv. *Bial Kalinkov*) were grown in a glasshouse in 5 L plastic pots on a commercial soil-peat substrate. Whole plants were treated with HT (35°, 40° and 45°C for 1, 2, 3, and 4 hours) in the bud formation-blossoming stage. It was established that the applied HT regimes influenced the pepper plants to varying extent. We concluded that: temperature values of 35 °C and 45 °C are not suitable to screen test pepper lines and varieties to heat tolerance; the male gamethophyte is more sensitive to HT stress compared with the photosynthetic apparatus; a semi-lethal effect on the male gamethophyte of pepper plants from the model cv. *Bial Kalinkov* was manifested at 40°C for 2 and 3 hours; the changes of the chlorophyll fluorescence parameters are better expressed at the higher values of HT regimes (40°C and 45°C for 2 and 3 hours); a suitable temperature regime to screen-test the heat tolerance of pepper species includes 40°C and duration of 2 or 3 hours.

Ключови думи: температурен стрес, селекция, фотосинтетичен апарат, мъжки гаметофит, хлорофилна флуоресценция.
Key words: temperature stress, breeding, photosynthetic apparatus, male gametophyte, chlorophyll fluorescence.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is among the main vegetable species in Bulgaria. The high temperatures (HT) (above 35°C), especially concurrent with the reproductive period of the plants, cause an abortion of their buds and flowers resulting in significant decrease of productivity (Veselinov et al., 1984). This has become a serious problem to pepper growers and breeders and the best way to solve it is by breeding temperature tolerant varieties (Aloni et al., 2001).

The photosynthetic apparatus (PSA) and male gametophyte have been considered as rather sensitive to

HT stress on plants (Nikolova et al., 2003), (Petkova et al., 2003, 2007, 2009). The analysis of the chlorophyll *a* fluorescence parameters is one of the fast contemporary methods for an assessment the physiological status of the plants in different environmental conditions (Berry and Björkman, 1980), (Strasser et al., 2005), (Zhang and Sharkey, 2009). Successful fruit set depends on several reproductive processes including pollen germination and tube growth processes (Erickson and Markhart, 2002), (Reddy and Kakania, 2007).

The heat stress tolerance is among the priority directions of the vegetable crops breeding programmes in the "Maritsa" Vegetable Crops Research Institute (MVCRI) in the recent years. Here we present the first stage of our work in the pepper breeding programme for create tolerant to high temperature varieties. The aim was to determine a critical temperature regime (values and duration of the temperature influence) appropriate for screen pepper breeding materials for high temperature tolerance.

MATERIALS AND METHODS

Experimental set-up

The experiment was carried out with model cultivar "Bial Kalinkov" belonging to the blocky type peppers from the collection of MVCRI. The plants were grown in plastic 5 L pots containing commercial soil-peat substrate in a greenhouse at day temperature of 23-25°C with the plant density of 3 plants per pot.

During the bud formation-blossoming period the whole plants were exposed to heat treatment (HT) in different regimes (values of 35, 40 and 45°C and treatment continuance 1, 2, 3 and 4 hours). The untreated plants were used as controls.

Chlorophyll fluorescence measurements

The PSA activity was characterized by analysis of the chlorophyll *a* fluorescence parameters, measured by a fluorimeter Plant Efficiency Analyser (PEA MK2, Hansatech Ltd, UK). The fluorescence parameters were registered minimum in 15 replicas, on intact, 30-min dark adapted, fully developed leaves, illuminated with actinic light (>650 nm), provided by an array of 6 light-emitting diodes (LED), focused on a leaf area of 4 mm diameter to produce homogeneous illumination with photon flux density (PFD) of 3000 μmol m^{-2} s^{-1}. A total measuring time of 5 sec was used throughout the experiments. The measurements were taken from all the plants before the HT treatment (Control) and immediately after the treatment (HT).

The data were statistically processed by the common MS Excel software.

Cytological analyses

The male gametophyte viability was characterized by three indices – presence of flowers with unviable pollen, pollen grain germination ($\bar{x}$, %) and pollen tube elongation (l, μm). The method of the hanging drop was used for the pollen grain germination. The pollen was sown in Petri dishes with nutritive medium containing agar, saccharose, H_3BO_3 and $CaCl_2$. The percentage of germination and the pollen tube lengths were determined 24 hours after placing the Petri dishes in a thermostat at 26°C. Two or three drops from each flower were used for the pollen germination with a minimum number of 100 pollen grains per drop counted. The pollen tube length was measured by eye-lens micrometer. The same parameters were studied in flowers of the control untreated plants.

RESULTS AND DISCUSSION

Chlorophyll fluorescence

The results obtained from the measurements the chlorophyll *a* fluorescence parameters are shown in Table 1. It can be seen that changes in chlorophyll fluorescence parameters have been exposed after a treatment with HT. Increased values for Fo and a reduced values for Fm were observed in treated with 40°C and 45°C. However, essential changes in Fo were not registered even in the plants treated with 40°C and percentage difference observed did not exceed 14%. The decrease of the Fm values was more

Таблица 1. Промени в параметрите Fo и Fm в растения от пипер, сорт "Бял Калинков", третирани с висока температура. Данните са осреднени от 15 измервания ± стандартно отклонение

Table 1. Changes in Fo and Fm parameters of pepper plants, cv. "Bial Kalinkov" treated with HT. Values represent the means of 15 measurements ± standard deviation

HT regimes	35°C		40°C		45°C	
	Fo±sd	Fm±sd	Fo±sd	Fm±sd	Fo±sd	Fm±sd
Control	240±11,4	1242±33,9	242±5,0	1236±55,9	232±7,9	1235±35,7
1 h	262±41,5	1376±130,5	243±13,0	1204±73,5	253±40,4	1213±136,1
2 h	259±34,8	1364±119,7	262±44,1	1172±100,3	257±25,9	1120±99,2
3 h	240±10,3	1310±113,7	259±20,8	1153±98,8	257±28,6	1078±109,6
4 h	247±12,1	1298±88,9	261±17,8	1104±93,7	264±17,0	1045±137,4

pronounced in the plants subjected to the strongest applied HT stress (45°C for 3 and 4 hours).

The ratios Fv/Fo and Fv/Fm are considered as indicators for the PS2 efficiency in primary photochemical reactions. The maximum quantum yield of primary photochemistry (Qy) was calculated using Fv/Fo ratio (Table 2). The plants treated with 40°C and 45°C in all durations showed decrease of Qy compared to the controls. The differences between Qy in untreated plants and Qy after the HT influence were relatively small in the 1 h durations both at 40°C and 45°C. The maximum reduction in Fv/Fo ratio (31.5%) was observed in HT regime 45°C for 4 h, following with HT 40°C for 4 h (21,6%). In spite of the relative constancy of the Fv/Fm ratio, the results obtained show the same tendency in the change of its values as in Fv/Fo (Table 2).

In contrast, the mild stress at 35°C in all durations did not cause considerable effect on the PSA of the measured pepper plants. The chlorophyll fluorescence parameters Fo and Fm as well as their ratios Fv/Fo and Fv/Fm, did not change significantly. The initial fluorescence Fo increased with 9.4% in the first two hours and after that decreased to almost the control values. The ratio Fv/Fm stayed almost equal to the controls and the Qy was slightly changed.

Male gametophyte

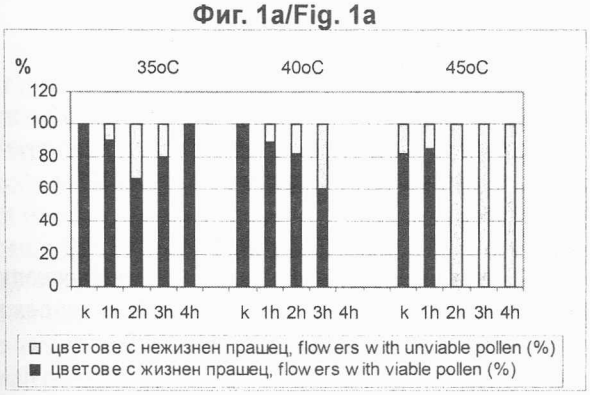
The temperature regime 35°C in all applied durations did not strongly influence the male gametophyte characteristics (Fig. 1a, b) and it is not suitable for a test of pepper gene pool. The increase of the temperature with 5°C (40°C) brought to an increase the percentage of flowers

Таблица 2. Промени в отношенията Fv/Fo и Fv/Fm в растения от пипер, сорт "Бял Калинков", третирани с висока температура. Данните са осреднени от 15 измервания ± стандартно отклонение

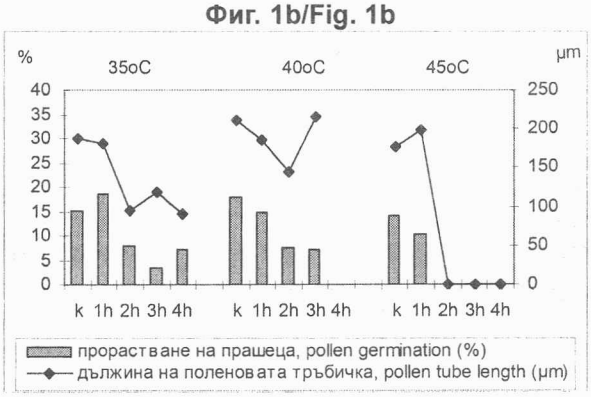
Table 2. Changes in Fv/Fo and Fv/Fm ratios of pepper plants, cv. "Bial Kalinkov" treated with HT. Values represent the means of 15 measurements ± standard deviation

HT regimes	35°C		40°C		45°C	
	Fv/Fm±sd	Fv/Fo±sd	Fv/Fm±sd	Fv/Fo±sd	Fv/Fm±sd	Fv/Fo±sd
Control	0,807±0,01	4,197±0,22	0,803±0,01	4,121±0,32	0,812±0,01	4,328±0,05
1 h	0,810±0,02	4,299±0,42	0,798±0,01	3,958±0,31	0,791±0,02	3,845±0,51
2 h	0,810±0,01	4,300±0,34	0,777±0,02	3,533±0,45	0,770±0,02	3,379±0,35
3 h	0,816±0,01	4,455±0,31	0,774±0,02	3,463±0,37	0,762±0,02	3,216±0,29
4 h	0,809±0,01	4,257±0,41	0,763±0,01	3,230±0,25	0,743±0,04	2,965±0,51

Фиг. 1a/Fig. 1a



Фиг. 1b/Fig. 1b



k - контролни растения, control plants

1h, 2h, 3h, 4h - продължителност на третиране с висока температура; continuance of HT treatment

Фиг. 1a, b. *Цветове (%) с жизнен и нежизнен прашец, прорастване на прашеца ($\bar{x}$, %) и дължина на поленовата тръбичка ($\bar{l}$, µm) в контролни и третирани с висока температура растения от пипер, сорт "Бял Калинков"*

Fig. 1a, b. *Flowers (%) with viable and unviable pollen, pollen germination ($\bar{x}$, %) and pollen tube length ($\bar{l}$, µm) in control and HT treated pepper plants, cv. "Bial Kalinkov"*

with unviable pollen (11.1, 18.2 and 40.0% at 1, 2, and 3 hours, respectively) (Fig. 1a). The HT treatment with 45°C in 2, 3, and 4 hours caused a lethal effect on the pollen viability. The values of pollen germination and pollen tube length were practically zero at the HT 45°C in 2, 3 and 4 h (Fig. 1b). The shown differences between the male gametophyte reactions of pepper plants to the various HT regimes allow us to prefer as suitable regime for an assessment of pepper breeding materials 40°C with duration 2 and 3 hours. This regime has a semi lethal effect and is able to disclose the available diversity in the pepper accessions on the basis of their pollen reaction to high temperature stress.

CONCLUSIONS

We conclude that the male gamethophyte is more sensitive to HT stress compared with the photosynthetic apparatus.

Temperature regimes of (1) 35°C with duration 1-4 hours and (2) 45°C with duration 2, 3, 4 hours, are not suitable for revealing genotype differences between the heat tolerance of pepper genotypes.

A semi lethal effect on the male gamethophyte of pepper plants from the model cv. "Bial Kalinkov" was established at temperature 40°C for 2 and 3 hours.

Temperature regime of 40°C with duration 2 and 3 hours is suitable to screen test for heat tolerance in the pepper gene pool for breeding purposes.

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