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High temperature effect on the male gametophyte and the photosynthetic activity of two *Capsicum annuum* L. cultivars

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Abstract

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Induced mutagenesis is imposed as one of the most powerful methods for creating a starting material in the selection. This also requires extensive research into mutant forms and their hybrid varieties. A prospective trend in selection is the creation of F_1 hybrids with high tolerance to abiotic stress. The high temperature (HT) is already a significant factor of the environment when growing different cultural plants and the pollen vitality and pollen fertility occur to be one of the most high temperature stress sensitive indicators. The effect of high temperature stress on two *Capsicum annuum* L. cultivars: Cv. Zlaten Medal 7 and cv. Yasen F_1, which was created on the basis of male sterility obtained by irradiating dry seeds with a range of Co^{60} frames, has been investigated.

In the bud formation-blossoming period the plants were exposed to high temperature treatment in two regimes – 40°C/2 h and 45°C/1 h. More sensitive to high temperatures was the male gametophyte of mutant cv. Yasen F_1 in comparison to cv. Zlaten medal 7 in which decisive role over the vitality occurred to be the treatment duration. According to the chlorophyll fluorescence analyses, the two HT regimes result in a change in the chlorophyll fluorescence parameters. The influence of the higher HT value with shorter impact duration is more pronounced. The mutant nature of Yasen F_1 may be the basis for its higher sensitivity to high temperatures.

Keywords: heat stress; pollen germination; pollen tube length; chlorophyll fluorescence; *Capsicum annuum* L.

Introduction

In a global climate warming, the reproductive period of the agricultural crops frequently coincides with heat stress (HS), which causes serious yield losses (Deryng et al., 2014; Gourdji et al., 2013). High temperature (HT) is an abiotic stress of vast importance over the growth and development of plants, with large effects on all levels and processes of plant metabolism. To reveal the dangerous impacts of HT stress and to combat them an integrated approach that requires collaborative efforts from various scientific disciplines is needed.

Male gametophyte and photosynthetic apparatus (PSA) are the very sensitive to abiotic stress systems of plants. Measurement of physiological and cytological traits assumes key place in view of ever-increasing precision of next-generation phenotyping assays.

Pepper is a main vegetable crop in Bulgaria, grown in open field and greenhouse conditions. The pepper yield is pliable to different environment influences. The high temperatures during flowering often have negative influence over the pollen fertility, pollen tube growth, pollination that leads to buds, flowers and fruits fall and decreased fruit number per pepper plant (Wien, 1997; Han et al., 1996; Usman et al., 1999; Aloni et al., 1991, 2001; Erickson & Markhart, 2002). Although both male and female gametophites are HT sensitive the male gametophite shows higher vulnerability, so at the HT the pollen germination and the pollen tube

growth degree are significantly decreased (Weaver 1989; Kakani et al., 2005).

High temperature affects the photosynthetic processes by its effect on the rate of chemical reactions and on structural organization (Pastenes& Horton, 1996). The *Chl* fluorescence emitted by higher plants upon illumination carries a lot of information about the structure and function of the photosynthetic apparatus (Pastenes& Horton, 1996; Strasser et al., 2010).

The most susceptible to abiotic stresses component of the photosynthetic machinery is photosystem II (PS II) (Nath et al., 2013). Abiotic stressors strongly influence parameters of PS II, fast fluorescence emissions which are successfully used as criteria of assessment to stress tolerance (Baker &Rosenqvist, 2004; Maxwell & Johnson 2000; Stirbet et al., 2001; Petkova et al., 2007; Yang et al., 2009).

In the last years in the Maritsa Vegetable Crops Research Institute (MVCRI) – Plovdiv, breeding programs have been developed for the identification of genotypes possessing tolerance to abiotic stress factors and increasing the tolerance of the basic vegetable crops (garden pea – Kalapchieva&-Petkova, 2004; Petkova et al., 2009a; bean – Nikolova et al., 2003; Petkova et al., 2003; Petkova et al., 2007, tomato – Petkova et al., 2009b and pepper – Petkova et al., 2010; Petkova et al., 2014).

The aim of this study was to evaluate the thermostability of the male gametophyte and PSA of the plants of two pepper cultivars – Yasen F_1 and Zlaten medal 7.

Materials and Methods

Plant material, cultivation and treatment

The experiments were conducted during 2012-2013 in the MVCRI, Plovdiv, Bulgaria with two pepper (*Capsicum annuum* L.) cultivars – Yasen F_1 and Zlaten medal 7 developed in the Institute.

The plants were grown in a 5.5 L pots on soil-peat substrate in greenhouse compartment at 22-24/18-20°C T day/night ± 1°C. During the bud formation-blossoming period the plants were exposed to high temperature (HT) treatment in two regimes (temperature values and treatment continuance) – 40°C/2 h и 45°C/1 h in thermostat and returned to recover at a control temperature of 23°C. The regimes were established as suitable ones to screen heat-tolerance of pepper genotypes in a previous experiment (Petkova et al., 2010). Fifteen plants per treatment from each cultivar were used. The untreated plants from each cultivar were used as controls.

Cytological analyses

For investigation of the pollen viability in vitro were used nutrition mediums made from 15% solution of sucrose to which was added boric acid (H_3BO_3), calcium chloride ($CaCl_2$) and 1g agar-agar. The pollen was sown by the hanging drop method in Petri dishes with diameter 8 cm in which 5 ml of water was put. Reporting was made after the Petri dishes stayed for 24 h in thermostat at T 26-28°C. The pollen viability was defined by two characteristics when observed with microscope – sprout pollen (x, %) and pollen tube length (l, μm) which is detected by means of ocularmicrometer.

Fluorescence measurements

The assessment of high temperature influence on PSA, particularly on PS II status, was performed through basic chlorophyll a fluorescence parameters (Fo, Fm and Fv) and their ratios measured at two high temperature regimes (40°C/2 h and 45°C/1 h).

Chlorophyll fluorescence was measured on the upper surface of the third fully expanded leaf from the top of the plants down, after 30 minutes dark adaptation. A fluorometer Plant Efficiency Analyzer (PEA, Hansatech Ltd., UK) was used. The details of measurements were described in our previous publications (Petkova et al., 2007; 2010).

The following fluorescence parameters were recorded: Fo, Fm – minimum and maximum dark adapted fluorescence yield, respectively; Fv – variable fluorescence (Fv = Fm – Fo). They were used to calculate the ratios Fv/Fo and Fv/Fm which are considered as indicators for the PSII efficiency in primary photochemical reactions. In addition, stability of the light-harvesting complex (LHCII) at high temperature (HT) was assessed by ratio Fo(23°C)/Fo(HT°C) and the tolerance of the photochemical activity and O_2-evolving system was evaluated by the changes in Fv(23°C)/Fv(HT°C) (Yordanov et al,. 1997).

The chlorophyll fluorescence measurements were taken from all plants immediately after the HT treatment. In addition, the measurements also were provided 3 h and 24 h after heat treatment to assess the plant's recovery capacity.

The results of the performed study represent means values ± sd from three independent series, each at least in 5 repeats. The data were statistically processed by the common MS Excel software and only differences with $P < 0.05$ were discussed.

Results and Discussion

Cytological analysis. During the conducted cytological analyses in 2012-2013 it was found that the temperature regimes of 40°C/2 h and 45°C/1 h influenced negatively over the male gametophyte and in a significant part of the plant reproductive organs the pollen loses its viability. The pollen fertility during 2012 in the treated plants decreases with 76.9% to 9.1% average and during 2013 from 61.7% to 0.0% (Figures 1 and 2).

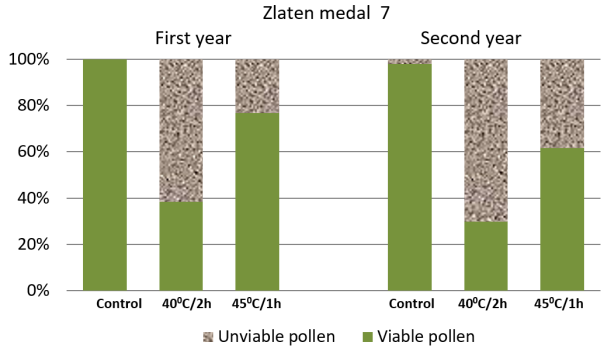

Figure 1. Buds and flowers (%) with viable and unviable pollen in plants of the cv. Yasen F1

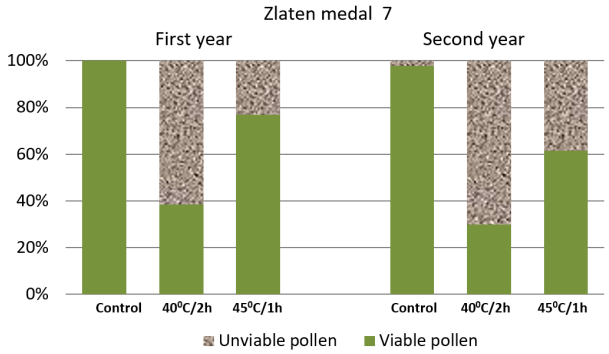

Figure 2. Buds and flowers (%) with viable and unviable pollen in plants of the cv. Zlaten medal 7

The temperature regimes 40°C/2 h and 45°C/1 h in the Yasen F_1 plants exerts exceptionally negative effect. During 2012 year, the average sprout percentage was with comparatively low values, 2.6 and 2.1 respectively and the pollen tube length 80.0 µm and 76.8 µm respectively was over two times lower than the reported in the control – 187.7 µm (Table 1). During the next year, the cv. Yasen F_1 sensitivity to high temperatures was more highly expressed and at 40°C it was observed high percentage of nonviable pollen – 90.6% and temperature over 45°C occurred to be critical for the viability indicators (Figure 1).The reason for this may be that Yasen was created on a male-sterile basis (Todorova&Arnaoudova, 2014).

Higher average values of the indicators pollen sprout and pollen tube length ($\bar{x}$ = 30.3% and $\bar{l}$ = 306.9 µm) were reported during the first year in the control plants of cv. Zlaten medal 7 in comparison with the cv. Yasen F_1 ($\bar{x}$ = 17.0% and $\bar{l}$ = 187.7µm) (Table 1). The tendency retains during the following year and from the analysis, it was clarified that more tolerant to the high temperatures is the male gametophyte of cv. Zlaten medal 7. When studying both indicators defining pollen viability (sprout and pollen tube length) it was found that in cv. Zlaten medal 7 during both years the values in the first treatment regime (40°C/2 h) were lower in comparison with those in the second (45°C/1 h). Therefore, decisive role over the viability of the male gametophyte in Zlaten medal 7 exerts the duration of the high temperature (Table 1, Figure2).

Table 1. Pollen viability (germination % and pollen tube length µm) in the control and in treated with high temperature plants

Year	Variants	Pollen germination (x%)			Pollen tube length (l µm)		
		x_{min}	x_{max}	Average x ± c	l_{min}	l_{max}	Average l ± c
Yasen F_1							
2012	Control	2.5	42.5	17.0 ± 12.5	84.5	356.8	187.7 ± 71.4
	40°C/2 h	1.0	5.2	2.6 ±1.7	54.9	131.8	80.0 ± 44.0
	45°C/1 h	1.0	3.1	2.1 ± 0.7	54.9	98.8	76.8 ± 23.6
2013	Control	1.0	48.4	17.1 ± 15.7	54.9	382.1	148.1 ± 85.4
	40°C/2 h	1.0	2.0	1.3 ± 0.4	120.8	164.7	139.1 ± 41.6
	45°C/1 h	0.0	0.0	0.0	0.0	0.0	0.0
Zlaten medal 7							
2012	Control	12.1	49.8	30.3 ± 11.1	126.3	531.4	306.9 ± 95.9
	40°C/2 h	1.0	8.9	3.3 ± 2.4	71.4	177.9	103.4 ± 55.2
	45°C/1 h	2.9	18.5	10.2 ±6.2	83.4	351.4	174.0 ± 99.6
2013	Control	1.0	68.4	25.5 ± 20.1	70.3	474.8	208.1 ± 101.1
	40°C/2 h	1.0	16.0	2.7 ± 2.5	62.5	109.2	76.6 ± 36.1
	45°C/1 h	1.0	18.7	3.0 ± 3.1	59.3	439.2	123.7 ± 85.5

Chl a fluorescence analysis. We compared the effect of HT stress on the PSA in pepper cultivars Yasen F_1 and Zlaten medal 7 by the changes in chlorophyll *a* fluorescence parameters at two high temperature modes. The obtained results are presented in Table 2 and Table 3. The data show also the chlorophyll *a* fluorescence parameters tracked in dynamics after the HT treatments.

The initial fluorescence (Fo) represents the minimal fluorescence level, when all reaction centers (RC) of PSII are open and the primary acceptor of electrons Q_A is fully oxidized. It is established that Fo increases under HT stress (Briantais et al., 1996). In the study, the temperature mode 40°C/2 h induced a slight increase in Fo (5 – 6.1% above the controls) in both cultivars and this trend was maintained 24 h after cessation of the temperature impact (Table 2). Under the impact of the mode 45°C/1h the initial Fo fluorescence was increased with 14% in

Table 2. Chlorophyll fluorescence parameters of dark-adapted intact peppers leaves treated under a heat stress of 40°C for 2 hours. In parentheses, percent to controls

Variants	Fo±sd	Fm±sd	Fv/Fm±sd	Fv/Fo±sd
Yasen F_1				
Control	492±18 (100%)	3212±89 (100%)	0.847±0.004 (100%)	5.539±0.23 (100%)
Immediately after the treatment	522±19 (106.1%)	2786±93 (86.7%)	0.812±0.006 (95.9%)	4.340±0.24 (78.3%)
Three hours after the treatment	521±21 (105.9%)	3006±94 (93.6%)	0.827±0.005 (97.6%)	4.778±0.23 (86.3%)
24 hours after the treatment	537±25 (109.1%)	3322±101 (103.4%)	0.838±0.013 (98.9%)	5.185±0.29 (93.6%)
Zlaten medal 7				
Control	523±19 (100%)	3215±92 (100%)	0.837±0.003 (100%)	5.149±0.22 (100%)
Immediately after the treatment	540±21 (105.0%)	2885±95 (90.0%)	0.813±0.005 (97.1%)	4.354±0.21 (83.0%)
Three hours after the treatment	531±28 (101.5%)	3237±89 (100.7%)	0.836±0.008 (99.9%)	5.107±0.28 (99.2%)
24 hours after the treatment	540±25 (103.2%)	3284±99 (102.1%)	0.835±0.010 (99.7%)	5.080±0.22 (98.6%)

Table 3. Chlorophyll fluorescence parameters of dark-adapted intact peppers leaves treated under a heat stress of 45°C for 1 hour. In parentheses, percent to controls

Variants	Fo±sd	Fm±sd	Fv/Fm±sd	Fv/Fo±sd
Yasen F_1				
Control	536±23 (100%)	3391±99 (100%)	0.842±0.004 (100%)	5.339±0.21 (100%)
Immediately after the treatment	611±39 (114.0%)	2646±79 (78.0%)	0.769±0.018 (91.3%)	3.349±0.30 (62.7%)
Three hours after the treatment	564±43 (105.2%)	2753±86 (81.2%)	0.795±0.019 (94.4%)	3.886±0.27 (72.5%)
24 hoursafter the treatment	577±42 (107.6%)	3481±81 (102.6%)	0.834±0.018 (99.0%)	5.041±0.25 (94.4%)
Zlaten medal 7				
Control	486±21 (100%)	3119±97 (100%)	0.844±0.004 (100%)	5.420±0.20 (100%)
Immediately after the treatment	585±41 (120.3%)	2807±81 (90.0%)	0.791±0.020 (93.7%)	3.805±0.31 (70.2%)
Three hours after the treatment	521±42 (107.2%)	3006±90 (96.4%)	0.827±0.017 (98.0%)	4.778±0.32 (88.2%)
24 hoursafter the treatment	544±39 (111.9%)	3407±78 (109.2%)	0.840±0.012 (99.5%)	5.269±0.35 (97.2%)

cv. Yasen F_1 and with 20.3% in cv. Zlaten medal 7 (Table 3). The registered elevated Fo values indicate that in the treated plants decrease the number of active RC of PSII.

The maximal fluorescence (Fm) presents Chl fluorescence emission when all the RC of PSII is closed and Q_A acceptors of PSII are in reduction form. Fluorescence quenching by nonphotochemical processes is expected under heat stress and may be expressed by a reduction in Fm at a damaging temperature (Krause & Weis, 1991). The registered Fm values in the experiment were lower in treated plants in both cultivars, showing significant decrease only in cv. Yasen F_1 at 45°C/1 h (22.0% below the control) (Table 3).In cv. Zlaten medal 7 the equal changes compared to controls were observed in both temperature regimes.

The Fv/Fo ratio represents the status and effectiveness of the electron transport chain. The plants of both cultivars subjected to 40°C/2 h and 45°C/1 h showed decrease of Fv/Fo compared to the controls. A trend toward bigger reduction in the F_1 hybrid cultivar compared to the direct one was observed at both temperature modes (Tables 2 and 3). Immediately after treatment with 40°C/2 h, Fv/Fo values decreased with 21.7% in cv. Yasen F_1 and with 17.0% in cv. Zlaten medal 7 (Table 2). The reaction of both cultivars was more pronounced at mode 45°C/1 h (Table 3). A maximal reduction in Fv/Fo ratio (37.3% below the control) was calculated in cv. Yasen F_1 at HT regime 45°C/1 h. The cv. Zlaten medal 7 showed greater effectiveness of the electron transport chain under high temperature conditions than the cv. Yasen F_1.

In the literature, Fv/Fm is used more frequently than Fv/Fo. The Fv/Fm ratio is considered as a measure of potential photosynthetic rates (Schreiber &Bilger, 1993) and as such should be a better indication of potential photosynthetic health. Temperature-dependent changes in Foand Fm determine different patterns of potential quantum efficiency of PSII (Fv/Fm). It can be seen, that after dark adaptation, both cultivars reacted with insignificantly decrease of Fv/Fm ratio under the temperature mode 40°C/2 h (4.1% and 2.9% below controls in cvs. Yasen F_1 and Zlaten medal 7, respectively) (Table 2). Following 3 h the Fv/Fm ratio in cv. Yasen F_1 increased to 97.6% compared to the control, and 24 h after the treatment it reached 98.9% towards to the control. The restoration ability of pepper plants after HT stress was more pronounced in cv. Zlaten medal 7.

A trend to decrease in Fv/Fm is observed when the temperature increased from 40 to 45°C, in spite of shorter duration of the higher temperature. As the results show (Table 3), immediately after the treatment the Fv/Fm ratio have been reduced with 8.7% and 6.3% in the Yasen F1 and Zlaten medal 7, respectively. The negative effect of heat stress on PSA is more pronounced again in the cv. Yasen F1, where Fv/Fm was reduced to 0.769 versus 0.791 in cv. Zlaten medal 7. In spite of significant reduction of Fv/Fm, the physiological status of the plants under the applied stresses remained in the physiological norm 0.75 and 0.85 (Bolhar-Nordenkampf&Oquist, 1993).

The established changes in the chlorophyll fluorescence parameters immediately after treatment do not give us grounds to characterize the tested pepper cultivars as heat-sensitive because of their plastic response. Both cultivars revealed a well-expressed recovery capacity. The Fv/Fm ratio increases only 3h after heat treatment, again more clearly marked in cv. Zlaten medal 7. In both cultivars 24 h after the treatment, the very stable parameter Fv/Fm equaled almost with the control values.

In interpreting chlorophyll fluorescence data, is difficult to distinguish between irreversible stress damage and temporary photoinhibition having a protective or adaptive function. Both can lead to a decreased Fv/Fm. Our results confirm the conclusion of Yamada (Yamada et al., 1996) that decline in Fv/Fm under stress usually involves an increase in Fo and both parameters are generally highly and negatively correlated. A reduction in *F*m was also correlated with the inhibition of photosynthetic activity due to heat stress, mainly to heat damage of the PSII complex (Krause & Weis, 1991).

Assessment of heat-dependent changes in minimum (Fo) and variable (Fv) fluorescence yield is an approach

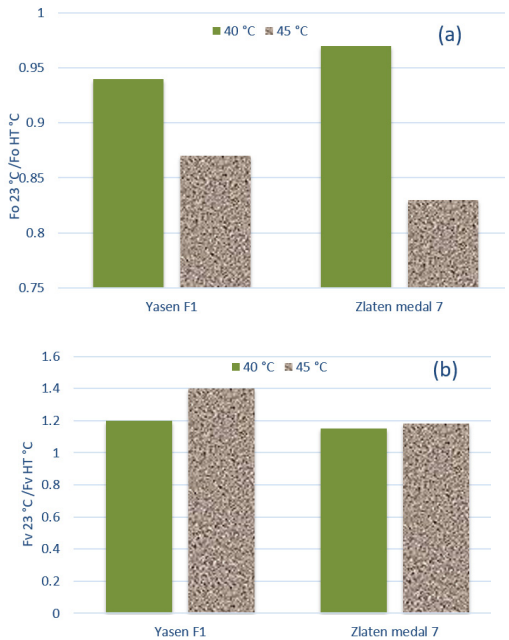

Figure 3. Ratio between chlorophyll fluorescence yields (minimum, Fo (*a*); and variable, Fv (*b*)) measured in dark adapted leaves of pepper cultivars at 23, 40 and 45°C

to estimate the photochemical apparatus under heat stress (Yordanovet al., 1997;Ribeiro et al., 2003). On Figure 3 are presented data obtained about the ratio Fo(23°C)/Fo(HT°C) giving information about stability of the light-harvesting complex (LHCII) at high temperature (Figure 3a) and the ratio Fv(23°C)/Fv(HT°C), characterizing the tolerance of the photochemical activity and O_2-evolving system (Figure 3b).

The ratio between Fo measured at 23 and 40°C did not show significant differences among tested cultivars, suggesting a similar thermal stability of the LHCII. The differences between the cultivars were more pronounced at the second temperature regime (45°C/1 h) (Figure 3a).

Comparison of the ratio of Fv(23°C)/Fv(45°C) reveals more significant differences between the two cultivars. As with the 40°C/2 h mode and the 45°C/1 h mode, the values of this ratio are higher for cv. Yasen F_1 (Figure 3b) which confirms its higher sensitivity to heat stress compared to cv. Zlaten medal 7.

Conclusions

The male gametophyte in cv. Zlaten medal 7 occurred to be more stable to high temperature stress in comparison with the one in cv. Yasen F1, in which decisive role over the vitality occurs to be the treatment duration.

The chlorophyll fluorescence parameters measured from the dark adapted leaves of pepper plants subjected under both HT regimes (40°C/2 h and 45°C/1 h), have been reacted with a reduction in Fm, the maximum quantum yield of PSII (Fv/Fm) and in electron transport rate (Fv/Fo), and with an increase in the initial fluorescence (Fo). The influence of the higher value and the shorter impact of the temperature factor are more pronounced.

According to the results obtained, adaptation ability was observed in both cultivars, more clearly expressed in cv. Zlaten medal 7.

The specific reaction of both male gametophytes to the applied temperature regimes allows the selection of a tolerant genotype that could be used in the process of breeding a high temperature stress resistance.

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