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EFFICIENCY OF PHOTOSYNTHETIC APPARATUS OF SWEET PEPPER (*Capsicum annuum* L.) F_1 HYBRIDS AND THEIR PARENTAL COMPONENTS IN HIGH TEMPERATURE CONDITIONS

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Abstract

The effect of high temperatures on the condition and functional activity of photosynthetic apparatus (PSA) in 12 sweet pepper (*Capsicum annuum* L.) genotypes – four F_1 hybrids and their parental forms, through the chlorophyll fluorescence parameters of photosystem II (PS II) and plastid pigments content in leaves was evaluated.

The study was conducted in 2010 – 2011 under field conditions. Measurements have been performed during the reproductive period of the plants, in the morning hours of the day at 21 – 23 ºC (used as controls) and in the afternoon at high temperature (33 – 35 ºC).

In the dark-adapted leaves, an increase of the initial (Fo) fluorescence and a decline of the maximum (Fm) and variable fluorescence (Fo), as well as the potential quantum efficiency of PS II (Fv/Fm) were observed in all genotypes at high temperature conditions.

In conclusion, it was considered that (I) photosynthetic apparatus of the F_1 hybrids possesses higher level of tolerance to moderate high temperature stress compared to that of the parental genotypes; (II) genotypes with orange fruits are more sensitive to high temperature than those with red-coloured fruits.

Key words: pepper (*C. annuum* L.), high temperature, tolerance, photosynthetic apparatus, chlorophyll fluorescence, pigments

Abbreviations: PSA - photosynthetic apparatus, PSII – photosystem II, RC - reaction centre, Chlorophyll – *Chl*, Q_A - primary acceptor of electrons; Fv/Fm - maximum quantum yield of the photosystem II photochemistry measured as the variable (Fv) to maximal (Fm) fluorescence ratio.

INTRODUCTION

High temperature stress is one of the most important abiotic stresses influencing productivity of vegetable species. Bulgaria is the first country in Europe in diversity of pepper genotypes, based on the longstanding traditions of pepper and overall vegetable growing (Tomlekova, 2010). The sweet pepper occupies 12.6% of the harvested areas of vegetables and the annual pepper production is 15.7% of the total production of vegetables in our country (Statistic of Ministry of Agriculture and Forest, 2011). Many of the local varieties have been spread to other European countries and are used as gene-carriers in the pepper breeding programs for developing new varieties in these countries (Timina et al., 2011).

The global climate changes in the recent decades, especially high temperature and drought, are a challenge for the pepper breeders. Their efforts have been aimed to enrich the genetic diversity in pepper species (Tomlekova et al., 2009) and to develop and access varieties from different types – conical, kapiya, blocky, red pepper, and directions - for fresh consumption, processing, grinding (Petkova & Todorova, 2001; Balkaya & Karaağaç, 2009; Todorova, 2011) with improved quality (Rêgo et al., 2011), high productivity potential (Luitel et al., 2011; Maaouia-Houimli et al., 2011), and toleran-ce to biotic (Petkova, 2006; McGregor et al., 2011) and abiotic stress factors (Petkova et al., 2007; 2010). High temperature values, especially coinciding with the reproductive period of the plants, cause negative influence on the physiological processes resulting in decrease of the photosynthetic activity and diminishing productivity of sweet pepper (Wang & Tseng, 2010).

The functional state of PSA has been considered as very useful physiological indicator for the sensitivity of the plants to stress factors (Richardson et al., 2002; Stoeva et al., 2010). *In vivo* chlorophyll *a* (*Chl*) fluorescence is widely used to study effects of different stresses on functional state of PSA (Vassilev et al., 2010; Zlatev et al., 2010). The analysis of the *Chl a* fluorescence parameters is one of the fast contemporary methods for assessment the physiological status of the plants in different environmental conditions (Strasser et al., 2005; Zhang & Sharkey, 2009). Synthesis of plastid pigments is of significant importance for the photosynthetic activity of plants. Plastid pigments content in normal and stress environmental conditions has been widely studied and discussed (Mikiciuk et al., 2010; Wrobel et al., 2010; Aienl et al., 2011).

Most of studies on the heat stress have been conducted in the greenhouses and growth chambers. The present study was aimed to evaluate the efficiency of PSA of sweet pepper genotypes – F_1 hybrids and their parental lines, under moderate high temperature stress by analysis of *Chl* fluorescence parameters and plastid pigments content under field conditions.

MATERIAL AND METHODS

<u>Experimental set-up</u>: The experiment was carried out in 2010 – 2011. The material for the present investigation comprised 12 genotypes – four F_1 hybrids and their parental mutant forms; with high fruit *β*-carotene

content (Tomlekova et al., 2006) from mutant collection recently developed under CRP12227 and CRP15406 IAEA projects. The pepper genotypes used are listed in Tables 1, 2.

The seeds were sown at the end of March (26 and 29, respectively) in unheated greenhouse on a peat-pearlitic substrate (1: 1). The seedlings were transplanted in the open field on 19th of May. The plants were grown according to the established technology for a mid-early production of sweet pepper. Fertilization, managing with weeds, diseases and pests, and other activities were implemented in due time in accordance with requirements of good protection practices.

Measurement of physiological traits: Determination of *Chl* fluorescence parameters (I) and total chlorophyll content (II) in pepper leaves as a part of the assessment of the heat sensitivity of their PSA was performed in triplicate during the reproductive period of the plants (the time of bud formation and flowering), which is considered especially critical for the plants. Measurements were taken for individual plant in field conditions, during mid-day on third-fourth intact leaves from the top of the plant. The twofold daily measurements of each analysis were applied to reach differences in the temperature values in the field conditions (respectively, morning at 21 – 23 ºC and afternoon at 33 – 35 ºC).

(I) *Chl* fluorescence parameters were measured *in vivo* using a Plant Efficiency Analyser (PEA, Hansatech Ltd., UK). The clips were attached on the upper (adaxial) surface of mature, fully developed leaves. The actinic light was provided by an array of 6 light emitting diodes (LED) (peak 650 nm), focused on the leaf sample area (4 mm diameter) to produce homogeneous illumination with photon flux density (PFD) of 3200 μmol $m^{-2}s^{-1}$ for 5 s (Strasser et al., 2005). Values represent the mean of the conducted 30 measurements (15 per year) ± standard deviation.

(II) Leaf chlorophyll contents were measured by a portable chlorophyll content meter CCM 200 (ELE Internat. Ltd., UK) on the same leaves and temperature conditions.

Statistical analysis: The SPSS program for Windows was used for the statistical analysis. Data presented are mean ± standard deviation of 30 replicates. The average data were compared by the least significance differences test (L.S.D).

RESULTS AND DISCUSSION

The summarized data from the *Chl* fluorescence parameters monitored during the pepper reproductive period - the initial *Chl a* fluorescence intensity (Fo), the maximum fluorescence intensity (Fm), and the ratios Fv/Fm and Fv/Fo, are presented in Table 1.

The data were analysed in terms of: (1) changes in *Chl* fluorescence parameters in different temperature conditions; (2) comparison of the registered values of *Chl* fluorescence parameters between F_1 hybrids and their parental forms.

The changes of values of *Chl* fluorescence parameters and their ratios in pepper plants at high temperature are well expressed as a percentage calculated to the values obtained at 21 – 23 ºC.

The initial fluorescence (Fo) represents the minimal fluorescence level, when all reaction centres (RC) of PS II are open and the primary acceptor of electrons Q_A is fully oxidized. It is established that the loss of the excitation energy during its transfer from the pigment bed to RC of PS II, expressed by Fo, increases under high temperature stress (Briantais et al., 1996). In our experiment an increase of initial *Chl* fluorescence was observed in all genotypes at high temperature conditions, but these responses were in a different extent between the genotypes. The greatest differences between Fo values at p.m. compared with a.m. measurements were observed in line 347/07 ♂, followed by line 2112/09 ♀ and 325/08 ♂. The Fo values at high temperature of these genotypes exceeded the relevant controls with 60.3, 57.8 and 39.6%, respectively. It is notable that all three genotypes are parental lines (two father's and one mother's) and that they are characterized with orange-coloured fruits. In comparing the temperature induced changes in the values of Fo in leaves of the F_1 hybrids and their parental lines one can be see that the Fo increase in the hybrids is less than that in the parental lines.

The maximal (Fm) fluorescence refers to *Chl* fluorescence emission when all the RC of PS II are closed and Q_A acceptors of PS II are in reduction form (Q_A^-). The registered Fm values are lower at high temperature in all studied genotypes. It is a result of fluorescence quenching. The lines 2112/09, 325/08, 298r/06, and 2106/09 revealed relatively small decreases in Fm values at high temperatures – between 7.0 and 9.1% lower compared with the controls. Dependence between the extent of the changes in Fm values as well as the fruit colour and the belonging of genotypes to F_1 hybrids or parental forms was not observed.

The simultaneous increase of Fo and decrease of Fm fluorescence reflected in decrease in the variable fluorescence Fv (Fv = Fm – Fo) upon the day course of temperature in all genotypes. The decrease in Fv values is considered as one of the indicators for reduced photochemical quantum conversion (Lichtenthaler, 1988). Therefore the maximum quantum yield of primary photochemistry (Qy) was calculated using Fv/Fo ratio. Fv/Fo indicates the status and effectiveness of the electron transport chain. The results showed lower values of this ratio under high temperature influence in all studied genotypes. Trend toward less reduction in the F_1 hybrids compared to the parental lines was observed.

The reduced potential of PS II activity (Fv/Fo) by the stress influences proves higher level of sensitivity to the stress than the maximum quantum efficiency of PS II primary photochemistry, expressed by the variable/maximum fluorescence ratio (Fv/Fm). A decrease in this parameter indicates down regulation of photosynthesis or photoinhibition. According to Bolhar-Nordenkampf & Oquist (1993) the ratio Fv/Fm in the plants with normal physiological status is between 0.75 and 0.85. Our results showed a slight decrease in this parameter in the most of the studied genotypes (Table 1). Fv/Fm was reduced in a greatest extent in the line 347/07 (7.1% to the control). Values of Fv/Fm in high temperature conditions under the biological minimum and close to it were registered in lines

Table 1. Chlorophyll fluorescence parameters of dark adapted intact pepper leaves measured by PEA fluorimeter (Hansatech, Ltd., UK) in reproductive period of plants in field conditions at 21 – 23 °C (controls) and at high temperature (33 – 35 °C). Presented values are the mean of 30 measurements ± standard deviation. In parentheses – per cent to controls.

Genotypes	Fruit colour	Fo	Fm	Fv/Fm	Fv/Fo
		($\bar{x}$ ± sd)	($\bar{x}$ ± sd)	($\bar{x}$ ± sd)	($\bar{x}$ ± sd)
At temperature 21 – 23 °C (controls)					
172/09 F_1	Red	582 ±21.08	3580 ± 125.13	0.838 ± 0.003	5.149 ± 0.10
298r/06 ♀	Red	593 ±26.50	3645 ± 136.53	0.837 ± 0.009	5.153 ± 0.34
2106/09 ♂	Red	605 ±17.74	3626 ± 95.30	0.834 ± 0.005	5.000 ± 0.19
172/08 F_1	Red	593 ±26.50	3645 ± 136.53	0.837 ± 0.009	5.153 ± 0.34
298r/06 ♀	Red	570 ±19.49	3488 ± 122.95	0.836 ± 0.011	5.125 ± 0.39
325/08 ♂	Orange	640 ±41.35	3352 ± 93.30	0.809 ± 0.013	4.257 ± 0.39
344/07 F_1	Red	585 ±11.16	3604 ± 17.15	0.838 ± 0.003	5.162 ± 0.12
345/07 ♀	Red	582 ±11.16	3543 ± 96.90	0.836 ± 0.007	5.093 ± 0.28
347/07 ♂	Orange	675 ±66.46	3253 ± 130.73	0.792 ± 0.025	3.862 ± 0.54
222/06 F_1	Orange	579 ±60.29	3222 ± 101.44	0.820 ± 0.021	4.625 ± 0.67
2112/09 ♀	Orange	705 ± 108.36	3316 ± 35.02	0.787 ± 0.034	3.783 ± 0.61
288a/06 ♂	Red	696 ± 78.79	3587 ± 106.42	0.806 ± 0.021	4.205 ± 0.52
At high temperature (33 – 35 °C)					
172/09 F_1	Red	624 ±18.4** (107.2)	3244 ± 111.4** (90.6)	0.807 ±0.01* (96.3)	4.200 ± 0.20* (81.6)
298r/06 ♀	Red	606 ±36.0* (104.2)	3035 ± 108.2* (84.8)	0.800 ± 0.01 (95.5)	4.017 ± 0.25** (78.0)
2106/09 ♂	Red	674 ±44.3* (115.8)	3254 ± 114.7* (90.9)	0.793 ± 0.01* (94.6)	3.846 ± 0.35** (76.9)
172/08 F_1	Red	606 ±36.0* (104.2)	3035 ± 108.2* (84.8)	0.800 ± 0.01* (95.5)	4.017 ± 0.25* (78.0)
298r/06 ♀	Red	654 ±37.0* (112.4)	3261 ± 106.4* (91.1)	0.799 ± 0.02* (95.4)	4.003 ± 0.38* (78.1)
325/08 ♂	Orange	813 ± 85.5** (139.6)	3307 ± 156.4* (92.4)	0.755 ± 0.02* (90.0)	3.093 ± 0.31** (72.7)
344/07 F_1	Red	632 ± 52.1** (108.6)	3284 ± 133.2* (91.7)	0.807 ± 0.01* (96.3)	4.215 ± 0.38* (81.7)
345/07 ♀	Red	682 ± 65.0* (117.2)	3247 ± 99.3* (90.7)	0.790 ± 0.02* (94.3)	3.796 ± 0.44** (74.5)
347/07 ♂	Orange	933 ± 87.0** (160.3)	3060 ± 135.3* (85.5)	0.695 ± 0.03* (82.9)	2.296 ± 0.25** (59.5)
222/06 F_1	Orange	640 ± 59.7* (109.9)	2829 ± 141.0* (79.0)	0.773 ± 0.03* (92.2)	3.459 ± 0.53* (74.8)
2112/09 ♀	Orange	918 ± 60.7* (157.8)	3328 ± 155.6* (93.0)	0.724 ± 0.02* (86.4)	2.635 ± 0.26* (69.7)
288a/06 ♂	Red	697 ± 74.2* (119.8)	3067 ± 117.2* (85.7)	0.772 ± 0.03* (92.2)	3.441 ± 0.51* (81.8)

* $P < 0.05$; ** $P < 0.01$.

347/07 (0.695), 2112/09 (0.724) and 325/08 (0.755). There is a tendency for smaller temperature-induced reduction of this ratio in the F_1 hybrids compared with that in the parental lines.

It is known that the pigments of photosynthesis present a main components of PSA and have a prime role not only in the life of the plants, but for existence of whole planet. The content of the chlorophyll pigments determines in a large extent the state and activity of the PSA and has considered as an indicator of different stress factors (Hendry & Grime, 1993). Our results showed that there was a significant variability of plastid pigment content in the investigated pepper genotypes (Table 2). The four F_1 hybrids (172/09 F_1, 172/08 F_1, 344/07 F_1 and 222/06 F_1) exceeded the better parent in synthesized total chlorophyll pigments. The values of the total pigments at the morning measurements in F_1 hybrids varied between 96.2 (222/06 F_1 hybrid) and 101.2 (172/08 F_1 hybrid) (CCM 200 value). The registered amounts of synthesized pigments in F_1 hybrids exceeded those in the parental lines also at high temperature. The reduction of the plastid pigments at high temperature conditions compared with these at morning temperature, were more pronounced in parental lines. A positive relationship between the total chlorophyll content in the leaves and the values of

Table 2. Total chlorophyll content in leaves of pepper (*Capsicum annuum* L.) genotypes – F_1 hybrids and their parental lines, measured by a portable chlorophyll content meter CCM 200 (ELE Internat., Ltd., UK) at reproductive period in field conditions at temperature 21 – 23 °C (controls) and high temperature (33 – 35 °C). Presented values are the mean of 30 measurements ± standard deviation. In parentheses – per cent to controls.

Genotypes	Fruit colour	CCM 200 value	
		at temperature 21 – 23 °C (controls)	at high temperature (33 – 35 °C)
172/09 F_1	Red	99,4 ± 13,1	98,3 ± 15,1* (98,9)
298r/06 ♀	Red	98,4 ± 10.2	95,1 ± 10,4* (96,7)
2106/09 ♂	Red	98,8 ± 9,9	94,4 ± 8,9 * (95,6)
172/08 F_1	Red	101,2 ± 12,3	103,3 ± 11,1* (102,1)
298r/06 ♀	Red	97,8 ± 11,0	93,9 ± 14,2** (96,0)
325/08 ♂	Orange	90,0 ± 10,1	82,1 ± 8,1** (91,2)
344/07 F_1	Red	100,2 ± 14,0	97,5 ± 13,2*(97,3)
345/07 ♀	Red	97,6 ± 11,7	94,4 ± 11,7* (96,7)
347/07 ♂	Orange	98.0 ± 16,7	89,2 ± 13,2** (91,0)
222/06 F_1	Orange	96.2 ± 12,0	93,6 ± 10,9* (97,3)
2112/09 ♀	Orange	91,7 ± 14,2	86,7 ± 12,1** (94,6)
288a/06 ♂	Red	93,3 ± 15,0	90,9 ± 14,7* (97,4)

* $P < 0.05$; ** $P < 0.01$.

initial *Chl* fluorescence (Fo) has established. Considering the obtained results, we support the Brown et al. (1991) and Hendry & Grime (1993) in their opinion, that the loss of pigments during environmental stress is a highly visible indicator of their destruction caused of diseases, mineral deficiencies, water shortage, temperature extremes, etc. The maximal differences in chlorophyll content of the stressed and control plants were established in lines 347/07 and 325/08. The both lines are paternal lines with orange coloured fruits. A trend of a bigger content of plastid pigments in the studied genotypes with red coloured fruits compared with orange ones as in normal, as well as in high temperature conditions, was observed. For instance, the lowest value – 90.0, at morning temperature measurements, was registered in parental line 325/08 ♂ with orange coloured fruits versus the hybrid 172/08 F_1 – 101.2 (with red colour fruits).

The chlorophyll content in most of the studied genotypes at high temperature has less value compared with the controls. All parental genotypes showed smaller quantity of the chlorophyll pigments under high temperature influence. In red-fruited genotypes the percentage of the chlorophyll content at high temperature in comparison with that in controls varied between 95.6-98.9% in the parents of 2106/09 and 172/09 F_1, respectively. The hybrid 172/08 F_1 with red fruits demonstrated higher value of pigment content at high temperature in comparison to that at morning temperature. A definite tendency to enhanced adaptive possibilities to unfavourable environmental conditions such as high temperatures of the red fruit accessions was observed. Our data confirm standpoints of other researchers. In a study with common bean plants, infected by common and halo blight, Berova et al. (2007) have observed a reduction of photosynthetic pigments content in infected leaves and suggested that the reduced total chlorophyll content was due on the activation of its enzymatic degradation in the stressed plants. According to Kaiser (1982), the decrease of photosynthetic pigments amount is due of disturbances of their biosynthesis and the enhanced destructive processes.

CONCLUSIONS

The result of this study provided evidence that there are genotypic differences in the studied excerpt of *C. annuum* species with respect to heat tolerance of PSA, expressed by the changes of *Chl* fluorescence parameters and chlorophyll content. The PSA of F_1 hybrids reveals a trend to better tolerance to high temperature in comparison with the parental lines. The genotypes with orange fruits are more sensitive to high temperature than these possessing red coloured fruits.

The obtained results are in correspondence with our preliminary expectations concerning the advantages of red F_1 hybrids in terms of the physiological characteristics. In this regard, identification of pepper varieties with high-temperature tolerance would be beneficial in both current and future climate conditions.

Considering the obtained results, the investigated parental combinations are appreciated as suitable donors in the future pepper breeding programme aimed at developing F_1 hybrids.

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Ефективност на фотосинтетичния апарат на F_1 хибриди и техните родителски компоненти от пипер (*Capsicum annuum* L.) при висока температура

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Резюме

Изследвано е въздействието на високата температура върху състоянието и функционалната активност на фотосинтетичния апарат (ФСА) при 12 генотипа сладък пипер (*Capsicum annuum* L.) – четири F_1 хибрида и техните родителски форми чрез параметрите на хлорофилната флуоресценция на фотосистема II (ФС II) и съдържанието на пластидните пигменти в листата.

Проучването е проведено през 2010 – 2011 г. при полски условия. Измерванията са извършвани през репродуктивния период на растенията в сутрешните часове на деня при температура 21 – 23 °С (контроли) и в следобедните часове при висока температура (33 – 35 °С). Получените резултати показват повишение на стойностите на началната (F_0) и понижение на максималната (F_m) и вариабилната (F_v) флуоресценция, както и потенциалната квантова ефективност на ФС II (Fv/Fm) в тъмнинно адаптирани листа.

Установено е, че (I) ФСА на F_1 хибридите притежава по-високо ниво на толерантност към умерен високотемпературен стрес в сравнение с родителските генотипи; (II) генотипите с оранжеви плодове са по-чувствителни към висока температура от тези с червено оцветени плодове.