Bulgarian Journal of Agricultural Science, 30 (No 4) 2024, 628–635

Impacts of heat stress on the photosynthetic apparatus and polen viability in green pepper cultivars (*Capsicum annuum* L.)

Elena Topalova*, Yanina Arnaoudova and Velichka Todorova

Agricultural Academy, "Maritsa" Vegetable Crops Research Institute, 4003 Plovdiv, Bulgaria
** Corresponding author:* elena_topalova@yahoo.com

Abstract

Topalova, E., Arnaoudova, Y. & Todorova, V. (2024). Impacts of heat stress on the photosynthetic apparatus and polen viability in green pepper cultivars (*Capsicum annuum* L.). *Bulg. J. Agric. Sci., 30*(4), 628–635

The effect of high temperature stress on photosynthetic apparatus and polen viability between two commercial sweet green pepper cultivars, Stryama and Milkana F_1, was investigated. The first genotype is the father parent of the second, which was developed on the bases of male sterility. During the bud formation and flowering periods, the plants were treated with high temperatures of 40 and 45°C for 2 and 1 h, respectively. Chlorophyll fluorescence, chlorophyll content, pollen germination, flower tube length, and correlation links between the studied parameters were measured.

The applied stress increased the initial (Fo) fluorescence in dark-adapted leaves and decreased the maximum (Fm) and variable (Fv) fluorescence. The quantum yield (Fv/Fm) of the PS II also decreased in stressed plants under two temperature treatments, with the highest increase at 45°C for 1 h. Results showed that the contents of chlorophyll in pepper leaves were significantly reduced when exposed to high temperature stress throughout the experiment. The photosynthetic efficiency of cultivar Milkana F_1 revealed a trend towards higher tolerance than cultivar Stryama. The cytological results of the pollen germination and the high percentage of viable pollen showed the presence of heterosis effect in Milkana F_1 cultivar in high temperature stress conditions.

Keywords: Capsicum annuum L.; high-temperature stress; chlorophyll fluorescence; male gametophyte; tolerance; correlation

Introduction

Pepper (*Capsicum annuum* L.) is one of the main economic vegetable crops, and its fruits have great nutritional value due to the high content of many antioxidant compounds, vitamin C, and carotenoids (Kim et al., 2016; Jang et al., 2020). Abiotic stress, such as salinity, drought, flooding, nutrient toxicity, wind, extreme temperatures, and radiation, reduces crop yields by 65 to 87% on average (Koyro et al., 2012; Bhandari et al., 2018).

Heat stress is an important abiotic factor that can severely limit the morphological, physiological, and biochemical characteristics of plants and fruits (Ortiz et al., 2008; Fumia et al., 2023). This can stop plant growth and development and lead to a big drop in crop yields. High temperatures, especially during the reproductive phase, negatively affect physiological processes, reducing photosynthetic activity and the reproductive ability of sweet peppers, leading to reduced yield (Zhou et al., 2018).

Agricultural productivity is seriously threatened by future global climate change, which is predicted to result in temperature increases of 1.5–5.8°C by 2100 (Rosenzweig et al., 2001; Vuuren et al., 2008). Breeding of high-temperature resistant cultivars is the best method to solve a problem that has become serious for pepper growers and breeders, regardless of the large number of studies (Mittler et al., 2012). The response of plants to high-temperature stress varies widely, depending on temperature, species, age, phase of plant growth, and the length of temperature exposure.

Chlorophyll fluorescence measurement is a non-invasive method that can provide insight into a plant's ability to tolerate environmental stress and the degree of its damage from adverse conditions (Kalaji & Guo, 2008; Kalaji et al., 2014). It has been applied to monitor the photosynthetic response to temperature stress in many plants because the photosystem II (PSII) complexes are thought to be the parts of the photosynthetic machinery that are most sensitive to heat. Its use to determine the photosynthetic response to temperature stress has been documented for many plant species, such as pepper (Petkova et al., 2010), common bean (Petkova et al., 2007), wheat (Sharma et al., 2014), and tomato (Zhou et al., 2018).

Plant photosynthetic activity is dependent on the synthesis of plastid pigments. Plastid pigment concentration has been thoroughly researched and debated in both normal and stressful environmental situations (Wrobel et al., 2010; Aienl et al., 2011).

In angiosperms, the male gamete (pollen) is particularly vulnerable to high temperatures at all stages of development, especially during microsporogenesis and the processes of pollination and fertilization. Male sterility and the impairment of pollen development have been the main factors in reduced seed set and productivity under high-temperature stress (Burke & Chen, 2015). Breeding strategies for pepper genotypes have been developed at the Maritsa Vegetable Crops Research Institute (MVCRI) in Plovdiv, Bulgaria. These strategies include gametophytic selection, which helps find heat stress-tolerant gametophytes (Nikolova et al., 2003; Petkova et al., 2010; Arnaoudova & Arnaoudov, 2020).

The aim of the study was to compare the responses of the Stryama cultivar and the hybrid cultivar Milkana F_1 to high temperature stress by evaluating some physiological (chlorophyll fluorescence and chlorophyll content), and cytological parameters (pollen grains germination and flower tube length).

Material and Methods

Plant material and growing conditions

Two sweet pepper cultivars (*Capsicum annuum* L.): Stryama and Milkana F_1 were tested under high temperature stress. The experiment was carried out at the 'Maritsa' Vegetable Crops Research Institute (MVCRI), Plovdiv. These genotypes are appropriate for early and mid-early open field production and also in cultivation facilities. Their fruits are used in the intermediate stage (green) and are suitable for fresh consumption and processing. It is important to notice that the Stryama cultivar is the father parent for the F_1 hybrid cultivar Milkana, which was created on a male sterile base (Todorova & Pevicharova, 2018).

Plants were grown in 5.5-L containers in a 1:1 peat-perlite mixture. Three plants were grown in each pot, and four pots per treatment were used for each accession. The plants were cultivated using mid-early sweet pepper technology as good protective practices were applied which required timely fertilization, weed, disease, and pest management.

During the budding-flowering phenophase, the plants were treated with high temperatures of 40°C for 2 h and 45°C for 1 h, according to the temperature regimes established in our previous studies (Petkova et al., 2010; Arnaoudova et al., 2020). Untreated plants were used as a control.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were performed on intact leaves on the day of the temperature treatments. The measurements were carried out on dark-adapted leaves using a leaf clip. Intact, dark-adapted leaves were measured after 30 min of dark adaptation. Measurement repeatability was 10 times. The chlorophyll fluorescence measurements were taken from all plants immediately after the high temperature treatment. Parameters of fluorescence emission induction curves of treated plants were compared with those of respective controls (untreated plants) of the same variants. As part of the physiological response of plants to high temperatures, the values of chlorophyll fluorescence were measured 3, 24, and 48 h after the temperature effect stopped.

The chlorophyll fluorescence parameters were measured immediately after dark adaptation using a Plant Efficiency Analyzer (PEA) (Hansatch Instrument, King's Lynn, England). The clips were attached to the upper (adaxial) surface of mature, fully developed leaves. Fluorescence parameters Fo , Fm, Fv, Fv/Fm (maximum quantum efficiency of photosystem II (PS II)), and Fv/Fo (PS II activity) were recorded.

Measurement of chlorophyll content

The concentration of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) in fresh leaf tissue was determined in three replications using 80% acetone extraction, according to Lichtenthaler (1987). The optical density of the extracts was evaluated spectrophotometrically.

Cytological analyses

For the investigation of pollen viability *in vitro* nutrition mediums made from a 15% solution of sucrose were used, to which were added boric acid (H_3BO_3), calcium chloride ($CaCl_2$) and 1g of agar-agar. The pollen was sown by the hanging drop method in petri dishes with a diameter of 8 cm, in which 5 ml of water was put. Reporting was made after the petri dishes stayed for 24 h in thermostat at temperature 26–28°C. The pollen viability was defined by two character-

istics when observed with microscope – sprout pollen (x, %) and pollen tube length (l, μm) which is detected by means of ocular micrometer.

The data were statistically processed using MS Excel software and represent means values ± SD. Correlation analysis was conducted to determine the relationships among fluorescense parameters: Fo, Fm, Fv, Fv/Fo, Fv/Fm, chlorophyll, carotenoids and their ratios, and cytological parameters: polen germination and pollen tube length.

Results and Discussion

Analysis of the chlorophyll a fluorescence parameters in the leaves of the studied pepper cultivars under normal temperature conditions indicated a good physiological state of the plants before treatment (Table 1). The initial fluorescence (Fo) describes the loss of the excitation energy during its transfer from the pigment bed to the reaction centre of photosystem II (PS II). It is reached immediately after the illumination of the dark adapted leaves.

As a result of high temperature treatment, there was a tendency to increase the initial values of fluorescence, which was more pronounced with higher temperature treatment. The negative effect of high-temperature stress on the photosynthetic apparatus of plants was confirmed. There was a tendency to increase the values of the initial fluorescence (Fo) for tested variants, which is in accordance with the dependence of this parameter on stress effects established by Gisbert-Mullor et al. (2021). The average value of the initial fluorescence Fo increased by 8.4% when the 45°C mode was used for 1 h immediately after the termination of high temperature exposure in the Stryama cultivar. Three hours after the end of treatment, higher Fo values were recorded compared to the control by 8.9% (Table 1).

The research data collected during the experiment show that both the maximum (Fm) and variable (Fv) fluorescence values go down when exposed to high temperatures. The tendency to decrease the values of Fm and Fv as a result of the high temperature treatment is more pronounced in the second temperature regime immediately after treatment (45°C for 1 h). The measured values of the two parameters in Stryama and Milkana F_1 were 6.4–10.7% lower at maximum fluorescence and 7.9–14.6% lower at variable fluorescence

Table 1. Changes of chlorophyll fluorescence parameters in plants of assessing cultivars treated under different temperature conditions. Values are represented as means ± standard deviation (SD) of ten biological replicates

Temperature regime	Fo ±SD	Fm±SD	Fv ±SD	Fv/Fm±SD	Fv/Fm±SD
Stryama					
Control	560±37	3276±257	2715±237	0.828±0.011	4.861±0.36
2 h–40 °C					
Immediately after the treatment	570±50	3129±247	2559±223	0.777±0.014	4.286±0.41
3 h after the treatment	592±63	3357±220	2765±193	0.782±0.015	4.475±0.45
24 h after the treatment	540±24	3391±91	2851±90	0.799±0.007	5.021±0.27
48 h after the treatment	668±82	3758±206	3090±157	0.823±0.016	4.677±0.47
1 h–45 °C					
Immediately after the treatment	607±65	2926±567	2319±596	0.781±0.071	3.902±1.13
3 h after the treatment	610±69	3191±578	2581±599	0.799±0.064	4.314±1.16
24 h after the treatment	520±17	3284±190	2764±187	0.799±0.009	5.055±0.37
48 h after the treatment	659±67	3771±204	3112±176	0.825±0.015	4.768±0.51
Milkana F_1					
Control	622±34	3719±193	3097±202	0.832±0.014	5.000±0.49
2 h–40 °C					
Immediately after the treatment	642±39	3682±133	3041±145	0.784±0.013	4.526±0.43
3 h after the treatment	586±41	3584±138	2998±142	0.795±0.012	4.886±0.44
24 h after the treatment	675±41	3876±140	3201±153	0.825±0.013	4.764±0.46
48 h after the treatment	617±43	3772±145	3155±149	0.836±0.013	5.143±0.47
1 h–45 °C					
Immediately after the treatment	628±34	3481±205	2853±216	0.778±0.016	4.333±0.45
3 h after the treatment	567±28	3385±147	2818±150	0.791±0.011	4.732±0.37
24 h after the treatment	661±36	3664±216	3003±227	0.819±0.017	4.562±0.48
48 h after the treatment	638±67	3791±132	3153±124	0.832±0.016	4.984±0.48

than in control plants, respectively. The established decrease in Fv values during treatment at 45°C is an indicator of damage to thylakoid membranes under the influence of stress. The variable fluorescence (Fv), which is the derivative of the initial (Fo) and maximum (Fm) fluorescence, is considered by some researchers to be a particularly important parameter in the selection of resistance to high temperatures.

Dark-adapted values of Fv/Fm reflect the potential quantum efficiency of PS II and have been used as an early indicator of heat stress on plant photosynthetic performance (Zhou et al., 2015). Regardless of the observed changes in the obtained fluorescence parameters, the ratio of Fv/Fm remains within the limits of the physiological norm of 0.750 to 0.850 and shows that the applied stress does not significantly affect the primary photochemical activity of the pepper plants. In our study, treatment at 40 and 45°C did not significantly change Fv/Fm values, but nevertheless, our data confirmed earlier studies of the results obtained on sweet pepper (Gisbert-Mullor et al., 2021) and tomato (Zhou et al., 2016). Values below the accepted physiological minimum were not found; they ranged from 0.777 to 0.784 in the treatment of 2 h at 40°C and from 0.778 to 0.781 in the treatment of 1 h at 45°C (Table 1).

The Fv/Fo ratio, which is used to measure the state and efficiency of the electron transport chain in photosynthesis processes, is a good way to find out how temperature stress affects the state of a plant's photosynthetic apparatus. Reduced values of this fluorescence parameter for the two cultivars indicate a violation of electron transfer in photosynthesis processes. The registered decrease in the efficiency of the electron transport processes in heat-treated plants was observed at cultivar Stryama compared to Milkana F_1 cultivar in the temperature modes of 1 h at 45°C. A maximal reduction in Fv/Fo ratio (19.7% below the control) was calculated in cultivar Stryama at the high temperature regime immediately after the treatment of 45°C for 1 h.

The scheme for studying the physiological response of plants to high temperatures was extended by measuring the fluorescence values of chlorophyll after 3, 24, and 48 h after termination of the temperature effect. The obtained results of the analysis of the changes in the fluorescence parameters of chlorophyll show that the recovery ability of the plants of the studied genotypes is well expressed. A one-way response to high temperatures in plants of the same cultivar was established, regardless of the application of different regimes and duration of exposure. In the Stryama cultivar, the lowest values for Fo were recorded 24 h after the treatment, and for all other chlorophyll fluorescence parameters, they were established immediately after the treatment, while for Milkana F_1, the lowest values for Fo, Fm, and Fv were established 3 h after treatment, and for the ratios Fv/Fm and Fv/Fo, they were established immediately after the treatment. The restorative power is more pronounced in the Milkana F_1.

The concentration of photosynthetic pigments is one of the indicators of stress under various stress influences. In Table 2, the results of the laboratory analyses for the content of the synthesised photosynthetic pigments in the plants before and after their treatment with high temperatures are presented.

Chlorophyll *a* is the pigment with the largest percentage in the chlorophyll complex of plants and plays a major role in their photosynthetic activity. The content of chlorophyll changes under the influence of temperature stress. The data show that the content of chlorophyll *a* in the studied cultivars

Table 2. Changes in chlorophyll content (mg g^{-1} fresh weight) in leaves in pepper cultivars under different temperature conditions. The data represent the means ± standard deviation (SD) of three replicates. In parentheses – per cent to control

Temperature regime	Chl *a*±SD	Chl *b*±SD	Chl *a*+Chl *b* ±SD	Carot.±SD	Chl *a*/Chl *b* ±SD	(Chl *a*+Chl*b*)/ Carot.±SD
Stryama						
Control	1.62±0.015	0.43 ±0.014	2.05±0.030	0.95±0.001	3.79±0.093	2.15±0.033
2 h–40ºC	1.48±0.021 (91)	0.43±0.007 (100)	1.90±0.028 (93)	0.75±0.010 (79)	3.48±0.007 (92)	2.53±0.004 (118)
1 h–45ºC	1.40±0.006 (87)	0.40±0.025 (95)	1.81±0.031 (88)	0.73±0.018 (77)	3.47±0.200 (91)	2.47±0.017 (115)
Milkana F_1						
Control	1.46±0.029	0.39±0.008	1.85±0.022	0.84±0.018	3.79±0.152	2.21±0.022
2 h–40ºC	1.42±0.014 (97)	0.39±0.011 (101)	1.81±0.025 (98)	0.82±0.019 (97)	3.62±0.066 (96)	2.22±0.022 (101)
1 h–45ºC	1.34±0.021 (92)	0.36±0.023 (93)	1.70±0.044 (92)	0.73±0.011 (87)	3.72±0.185 (98)	2.33±0.025 (106)

is higher at normal temperatures, i.e., before exposing plants to high temperatures. The values of this pigment before treatment ranged from 1.46 in Milkana F_1 to 1.62 in Stryama and decreased after being exposed to high temperatures. The degree of the decrease found varies within insignificant limits, with a slight tendency for the test mode of 1 h at 45°C to have a stronger effect than the mode of 2 h at 40°C in Stryama cultivar.

A significant decrease in the content of carotenoids was observed in the Stryama cultivar at both temperature durations by 20.9 and 23.3%, while in the Milkana cultivar the decrease was only 2.6 and 12.8% compared to the controls. There is a trend towards compliance between changes in chlorophyll *a* content and photosystem II efficiency in stressed plants. The data obtained by us confirm the results of Zhou et al. (2015) for a decrease in the content of chlorophyll *a*, chlorophyll *b*, and carotenoids in tomato plants subjected to heat stress.

The chlorophyll *a*/*b* ratio is widely used as an indicator for early senescence. Our results show a decrease in the values of the chlorophyll *a*/*b* ratio, which is more pronounced in the Stryama cultivar at both treatment durations. The ratio between chlorophylls and carotenoids is a sensitive indicator for distinguishing timely natural ageing from ageing caused by environmental stress factors. In our experiment, we found an increase in the ratio of chlorophylls to carotenoids, which was more significant in the Stryama cultivar. The excess over the controls was 17.5% at 2 h 40°C and 14.9% at 1 h 45°C. Rajamedov et al. (2021) reported a similar decrease in chlorophyll content in the chlorophyll *a*/*b* ratio in hot peppers subjected to heat stress.

During the cytological analysis, it was found that the temperature regimes of 40°C for 2 h and 45°C for 1 h influenced the male gametophyte in both studied genotypes differently. Among the Stryama cultivar's reproductive plant organs, the pollen lost some of its vitality, and on average, the treated plants' pollen fertility fell by up to 40.0%. When analyzing the anther fertility of the Milkana F_1 hybrid, it was found to have a heterosis effect as the male gametophyte of the hybrid showed extreme tolerance towards the high temperature, and in all studied plants in both temperature regimes, the pollen fertility was 100% (Figure 1).

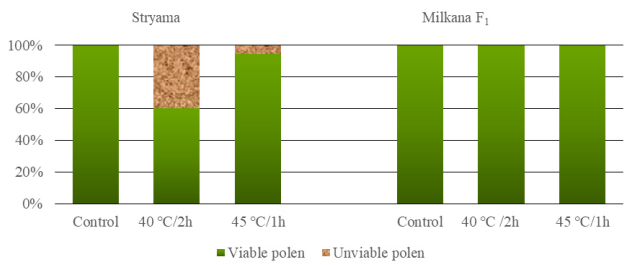

Fig. 1. Buds and flowers with viable and unviable pollen (%) in plants of the cv. Stryama and Milkana F_1

Extremely negative influences made the temperature regime 40°C 2 h and 45°C 1 h in the plants of Stryama cultivar. The average percentage of germination was relatively low at 8.5 and 4.0, respectively, and the length of the pollen tube was 127.0 µm and 177.1 µm, respectively, which is almost twice the value reported in the control 215.67 µm) (Table 3). The sensitivity of the Stryama cultivar towards high temperatures was highly expressed in 40°C, where it was reported 40.0% nonviable pollens, and temperature from 45°C occurred to be critical for 5.5% of the pollen only (Figure 1). That could also be confirmed by the elongation of the pollen tubes. When the temperature was 40°C the pollen tubes were shorter than these at 45°C where the maximum longitude in some tubes reached these from the controls (Table 3). Therefore, the longitude of the high temperature plays a key role in fertility and pollen viability in the Stryama cultivar. Similar results were reported in one of our previous studies on the Zlaten medal 7 cultivar (Arnaoudova et al., 2020).

Increased tolerance of the plants from the Milkana F_1 hybrid towards temperature stress was also reported in the characteristics defining pollen viability. The pollen germination and the length of the pollen tubes in the hybrid showed values twice as high as those in the father-parent Stryama cultivar 39.5% and 568.3 µm, respectively. The cytological results of the pollen viability confirm the heterosis effect of the Milkana F_1 hybrid. It gives the impression that the indexes for pollen viability in Milkana F_1 plants decrease regularly with increasing temperature as the germination decreases with 53.9% and 58.2% average in both regimes and the elongation with 44.5% and 47.1%. Therefore, we could assume that the sensitive genotype (in that case, the Stryama cultivar) expressed a stronger negative reaction towards the duration of the treatment, while the tolerant genotype (in that case, the Milkana F_1 hybrid) was more adaptive. Probably the comparative better results in pollen viability parameters for cultivar Milkana F_1 towards high temperature stress may be explained with its hybrid nature. Similar better results of this cultivar compared with his father component (Stryama) were reported for economic and fruit morphological characters (Todorova & Pevicharova, 2018).

Our data confirm the results obtained by Erickson & Markhart (2002) that high temperatures during flowering in pepper reduce pollen germination, pollen tube. Male gametophyte has been reported to be especially sensitive to severe temperatures, particularly temperatures above 30°C.

Table 3. Pollen viability (germination % and pollen tube length µm) in the control and in treated with high temperature plants of assessed cultivars

Variants	Pollen germination			Pollen tube length		
	x_{min} %	x_{max} %	$\bar{x}$ %	l_{min} µm	l_{max} µm	$\bar{l}$ µm
Stryama						
Control	5.0	24.0	14.4	73.6	416.1	215.7
2 h–40°C	0.8	23.5	8.5	65.9	239.4	127.0
1 h–45°C	1.0	24.2	4.1	72.5	410.6	177.1
Milkana F_1						
Control	19.1	55.0	39.5	222.9	1043.1	568.3
2 h–40°C	5.9	44.2	18.2	157.0	439.2	315.5
1 h–45°C	2.7	34.4	16.5	98.8	518.3	300.7

The results of the correlation study demonstrated significant strong to very strong positive links (the correlation coefficient was from 0.821 to 0.969) between polen germination (PG), pole tube lenght and the indicators Fm, Fv (Table 4). It was established that there was an almost functional positive correlation between the fluorescence parameters Fm and Fv ($r = 0.996$, $p < 0.01$), followed by Chl *a*+*b* and Chl *a* ($r = 0.991$, $p < 0.01$), who also correlated with Chl *b* ($r = 0.869$, $p < 0.05$).

There was a significant or proven strong positive correlation between Fv/Fm and Fv/Fo ($r = 0.867$, $p < 0.05$), while between Fo with Chl *b* and Chl *a*+*b*, it was very strong inversely proportional ($r = – 0.851$, $p < 0.05$) and ($r = – 0.825$, $p < 0.05$). The determined correlation links could be useful in the future pepper breeding programs.

Conclusion

The obtained results for the values of the main parameters and ratios of chlorophyll fluorescence, both immediately after the treatment and when following the restorative ability of the plants from Stryama and Milkana, follow a unidirectional tendency for the period of the study. In both studied cultivars, the temperature regime, including a higher temperature value with a shorter duration of impact, affected the fluorescence emission parameters to a greater extent.

As a result of the cytological study over the reaction of pollen fertility and viability in the conditions of two temperature regimes of 40°C/2h and 45°C/1h, we could conclude that the plants sensitivity was defined to a greater degree by the duration of the treatment and the thermotolerance by the temperature factor. When analyzing the pollen fertility in

Table 4. Correlation analysis between diferent physiological and cytological traits of studied pepper cultivars

	Fo	Fm	Fv	Fv/Fm	Fv/Fo	Chl. *a*	Chl. *b*	Chl. *a*+*b*	Car.	Chl. *a*/*b*	Chl. *a*+*b*/*c*	PG	PTL
Fo	1												
Fm	0.613	1											
Fv	0.542	0.996**	1										
Fv/Fm	-0.245	0.368	0.418	1									
Fv/Fo	0.065	0.705	0.757	0.867*	1								
Chl. *a*	-0.782	-0.131	-0.053	0.683	0.564	1							
Chl. *b*	-0.851*	-0.561	-0.503	0.199	0.026	0.797	1						
Chl. *a*+*b*	-0.825*	-0.229	-0.153	0.602	0.467	0.991**	0.869*	1					
Car.	-0.403	0.228	0.350	0.829*	0.801	0.868*	0.437	0.805	1				
Chl. *a*/*b*	0.078	0.672	0.705	0.769	0.860*	0.342	-0.295	0.215	0.685	1			
Chl.*a*+*b*/*c*	-0.198	-0.716	-0.740	-0.729	-0.831*	-0.391	0.179	-0.281	-0.796	-0.885*	1		
PG	0.397	0.838*	0.847*	0.676	0.807	0.041	-0.410	-0.056	0.346	0.714	-0.635	1	
PTL	0.570	0.821*	0.810	0.588	0.675	-0.137	-0.558	-0.234	0.218	0.655	-0.605	0.969**	1

Fo – initial fluorescence, Fm – maximum fluorescence, Fv – variable fluorescence, Fm/Fv – maximum quantum efficiency of photosystem II and Fv/Fo – (PSII activity); Chl. – chlorophyll; Car. – carotenoids; PG – pollen germination and PTL – pollen tube length

$p < 0.05$; ** $p < 0.01$; $p < 0.001$

the cultivar Milkana F_1, a heterosis effect was found. The male gametophyte of the hybrid showed extreme tolerance towards the high temperatures of both regimes, with pollen fertility up to 100% and twice higher values for pollen viability parameters compared with its father component, the Stryama cultivar.

The specific reactions of the two cultivars to the applied temperature regimes allow the selection of a tolerant genotype to be used in the selection process for resistance to high temperature stress. The established strong, very strong, and functional correlation links between some studied parameters could be useful in the future pepper breeding programmes.

References

Aienl, A. S., Khetarpal, M. & Pal, C. (2011). Photosynthetic characteristics of potato cultivars grown under high temperature. *American-Eurasian J. Agric. & Environ. Sci*., *11*(5), 633-639.

Arnaoudova, Y. & Arnaoudov, B. (2020). Screening *Capsicum* genotypes for increased drought tolerance by in vitro pollen germination and pollen tube length. *Trakia Journal of Sciences*, *1*, 52-58. https://doi.org/10.15547/tjs.2020.01.010

Arnaoudova, Y., Topalova, E. & Todorova, V. (2020). High temperature effect on the male gametophyte and the photosynthetic activity of two *Capsicum annuum* L. cultivars. *Bulg. J. Agric. Sci., 26*(2), 409–415. https://www.agrojournal.org/26/02-18.html.

Bhandari, S. R., Kim, Y. H. & Lee, J. G. (2018). Detection of temperature stress using chlorophyll fluorescence parameters and stress-related chlorophyll and proline content in paprika (*Capsicum annuum* L.) seedlings. *Horticultural Science and Technology*, *36*(5), 619-629. https://doi.org/10.12972/kjhst.20180062.

Burke, J. J. & Chen, J. (2015). Enhancement of reproductive heat tolerance in plants. *PLoS ONE 10*(4), e0122933. https://doi.org/10.1371/journal.pone.0122933.

Erickson, A. N. & Markhart, A. H. (2002). Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. *Plant, Cell & Environment*, *25*(1), 123-130. https://doi.org/10.1046/j.0016-8025.2001.00807.x.

Fumia, N., Kantar, M., Lin, Y.-P., Schafleitner, R., Lefebvre, V., Paran, I., Börner, A., Diez, M. J., Prohens, J., Bovy, A., Boyaci, F., Pasev, G., Tripodi, P., Barchi, L., Giuliano, G. & Barchenger, D. W. (2023). Exploration of high-throughput data for heat tolerance selection in *Capsicum annuum*. *The Plant Phenome Journal*, *6*(1), e20071. https://doi.org/10.1002/ppj2.20071.

Gisbert-Mullor, R., Padilla, Y. G., Martínez-Cuenca, M. R., López-Galarza, S. & Calatayud, A. (2021). Suitable rootstocks can alleviate the effects of heat stress on pepper plants. *Scientia Horticulturae*, *290*, 110529. https://doi.org/10.1016/j.scienta.2021.110529.

Jang, G. W., Choi, S. I., Han, X., Men, X., Kwon, H. Y., Choi, Y. E., Park, M. H. & Lee, O. H. (2020). Method validation and measurement uncertainty determination of ethoxyquin and antioxidant activity in paprika seasonings and paprika sauces frequently consumed in South Korea. *Separations*, *7*(4), 50. https://doi.org/10.3390/separations7040050.

Kalaji, M. H. & Guo, P. (2008). Chlorophyll fluorescence: a useful tool in barley plant breeding programs. *Photochemistry Research Progress*, *29*, 439-463.

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., Cetner, M. D., Łukasik, I., Goltsev, V. & Ladle, R. J. (2014). The use of chlorophyll fluorescence kinetics analysis to study the performance of photosynthetic machinery in plants. In: *Emerging Technologies and Management of Crop Stress Tolerance*; *Elsevier:* Amsterdam, The Netherlands, 347–384. ISBN 978-0-12-800875-1 https://doi.org/10.1016/B978-0-12-800875-1.00015-6.

Kim, J. S., An, C. G., Park, J. S., Lim, Y. P. & Kim, S. (2016). Carotenoid profiling from 27 types of paprika (*Capsicum annuum* L.) with different colors, shapes, and cultivation methods. *Food Chemistry*, *201*, 64-71. https://doi.org/10.1016/j.foodchem.2016.01.041.

Koyro, HW., Ahmad, P. & Geissler, N. (2012). Abiotic Stress Responses in Plants: An Overview. In: *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change; Springer:* New York, NY, 1-28. ISBN 978-1-4614-0815-4 https://doi.org/10.1007/978-1-4614-0815-4_1.

Lichtenthaler, H. K. (1987). Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods Enzymol.*, *148*, 350-382. https://doi.org/10.1016/0076-6879(87)48036-1.

Mittler, R., Finka, A. & Goloubinof, P. (2012). How do plants feel the heat? *Trends Biochem. Sci., 37*, 118–125.

Nikolova, V., Petkova, V. & Poryazov, I. (2003). Possibilities for selection of garden bean (*Phaseolus vulgari*s L.) genotypes tolerant to high temperature. Variation of pollen viability. *Annual Report of Bean Improvement Cooperative*, *46*, 83-84.

Ortiz, R., Sayre, K. D., Govaerts, B., Gupta, R., Subbarao, G. V. & Ban, T. (2008). Climate change: can wheat beat the heat? *Agric. Ecosyst. Environ., 126*, 46-58. https://doi.org/10.1016/j.agee.2008.01.019.

Petkova, V., Denev, I., Cholakov, D. & Porjazov, I. (2007). Field screening for heat tolerant common bean cultivars (*Phaseolus vulgaris* L.) by measuring of chlorophyll fluorescence induction parameters. *Scientia Horticulturae*, *111*(2), 101-106. https://doi.org/10.1016/j.scienta.2006.10.005.

Petkova, V., Nikolova, V., Todorova, V., Stoeva, V. & Topalova, E. (2010). Response of the photosynthetic apparatus and male gametophyte of pepper plants (*Capsicum annuum* L.) to various high temperature regimes. *Agricultural Sciences, II*(4), 89 – 92, Agricultural University – Plovdiv. https://doi.org/10.22620/agrisci.2010.04.015.

Rajametov, S. N., Yang, E. Y., Cho, M. C., Chae, S. Y., Jeong, H. B. & Chae, W. B. (2021). Heat-tolerant hot pepper exhibits constant photosynthesis via increased transpiration rate, high proline content and fast recovery in heat stress condition. *Sci. Rep.*, *11*(1), 14328. https://doi.org/10.1038/s41598-021-93697-5.

Rosenzweig, C. A., Iglesias, X. B., Yang, P. R., Epstein, E. & Chivian, E. (2001). Climate change and extreme weather events. Implications for food production, plant diseases and

pest. *Global Change and Human Health*, *2*, 90–104. https://doi.org/10.1023/A:1015086831467.

Sharma, D. K., Fernandez, J. O., Rosenqvist, E. & Ottosen, C. O. (2014). Genotypic response of detached leaves versus intact plants for chlorophyll fluorescence parameters under high temperature stress in wheat. *J. Plant Physiol.*, *171*, 576-586. https://doi.org/10.1016/j.jplph.2013.09.025.

Todorova, V. & Pevicharova, G. (2018). Milkana – new Bulgarian pepper variety (*Capsicum annuum* L. ser. var. *longum* Sendt.). *Bulgarian Journal of Crop Science*, *55*(3) 47-53.

Vuuren, D. P. V., Meinshausenc, M., Plattnerd, G. K., Joose, F., Strassmanne, K. M. & Smithg, S. J. (2008). Temperature increase of 21st century mitigation scenarios. *Proc. Natl. Acad. Sci., 105*(40), 15258–15262. https://doi.org/10.1073/pnas.0711129105.

Wrobel, J., Mikiciuk, M., Malinowska, K. & Drozd, A. (2010). Physiological reaction of *Salix viminalis* to stress of anthropogenic origin. *Agricultural Sciences*, *2*(4), 33-36. https://doi.org/10.22620/agrisci.2010.04.004.

Zhou, R., Yu, X., Kjær, K. H., Rosenqvist, E., Ottosen, C. O. & Wu, Z. (2015). Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. *Environmental and Experimental Botany*, *118*, 1-11. https://doi.org/10.1016/j.envexpbot.2015.05.006.

Zhou, R., Kjaer, K. H., Rosenqvist, E., Yu, X., Wu, Z. & Ottosen, C. O. (2016). Physiological response to heat stress during seedling and anthesis stage in tomato genotypes differing in heat tolerance. *Journal Agronomy & Crop Science*, *203*, 68–80. https://doi.org/10.1111/jac.12166.

Zhou, R., Wu, Z., Wang, X., Rosenqvist, E., Wang, Y., Zhao, T. & Ottosen, C. O. (2018). Evaluation of temperature stress tolerance in cultivated and wild tomatoes using photosynthesis and chlorophyll fluorescence. *Hortic. Environ. Biotechnol., 59*, 499-509. https://doi.org/10.1007/s13580-018-0050-y.

Received: **September, 26, 2023**; *Approved*: **January, 03, 2024;** *Published:* **August, 2024**