UDC 575.630
https://doi.org/10.2298/GENSR1902417K
Original scientific paper

MORPHOLOGICAL, PHYSIOLOGICAL AND PRODUCTIVITY RESPONSE IN GARDEN PEA GENOTYPES DURING HIGH TEMPERATURE STRESS

Slavka KALAPCHIEVA*, Elena TOPALOVA, Valentina PETKOVA

Department of Breeding, Variety Maintenance and Introduction, Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria

Kalapchieva S., E. Topalova, V. Petkova (2019): *Morphological, physiological and productivity response in garden pea genotypes during high temperature stress*.- Genetika, Vol 51, No.2, 417-428.
Changes in morphological characteristics, productivity and fluorescence emission parameters at normal and high temperature values under field conditions of 10 varieties and lines of the species *Pisum sativum* L. from the collection of MARITSA Vegetable Crops Research Institute were established. The influence of high temperature on the condition and functional activity of the photosynthetic apparatus (PSA) was studied by analyses of chlorophyll fluorescence characteristics of photosystem II (PSII) in the pea leaves. Plants were subjected to stress from high temperature during the reproductive period (bud formation - flowering phases), which is considered particularly critical for pea plants. The morphological performances and productivity have been influenced by climatic characteristics of the years. The screening for thermostability of the photosynthetic apparatus to the high temperature characterizes Skinado, Faboundo and line 95/4 as the most tolerant on the basis of analysis of fluorescence induction parameters. The most sensitive are lines 72/2 and 72/9. Varieties of Skinado and Fabundo respond with higher yields of green pods and grains in the conditions of the first year, categorized as hot compared to 2013. We consider that the indicated accessions are suitable for the pea-breeding programme to high temperature stress tolerance.
Keywords: pea, breeding, abiotic stress, reproductive period, yield

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Corresponding author: Slavka Kalapchieva, Department of Breeding, Variety Maintenance and Introduction, Maritsa Vegetable Crops Research Institute, Brezovsko shosse Str. 32, 4003 Plovdiv, Bulgaria, Tel.:+35932952296, Fax:+35932960177, e-mail: s_kalapchieva@abv.bg

INTRODUCTION

Globally the yields of garden pea are low and unstable mainly due to biotic and abiotic stresses. The most common abiotic stresses affecting the production of pea are drought accompanied by heat and cold (SINGH and SAXENA, 1993; MUEHLBAUER and KAISER, 1994; BENJAMIN and NIELSEN, 2006; CEYLAN *et al.*, 2006; CATTIVELLI *et al.*, 2008, TOKER and MUTLU, 2011).

The high temperature stress is common in major pea growing areas around the world, and occurs together with drought in many environments (MCDONALD and PAULSEN, 1997). The drought and high-temperature stresses caused yield losses of 21–54% in India, Syria and New Zealand (SAXENA *et al.*, 1993).

Interaction of these stresses often coincides with the phase of reproductive development in peas and reduced the duration of flowering and pod filling, caused withering and burning of lower leaves, desiccation of poorly developed plants, stunting of flowers and pod abortion, and reduced root nodulation and nitrogen (N) fixation, resulting in large yield losses (SAXENA *et al.*, 1988; VAN RHEENEN *et al.*, 1997). Flowers are the organs most sensitive to heat (TOKER and YADAV, 2010).

In Bulgaria garden peas are not usually irrigated in the northern parts of the country. South Bulgaria was characterized by high temperatures, accompanied by drought, which reduces the yield and overall quality of pea, thereby contributing to a considerable reduction in total pea area in the last years.

A negative relationship between the high temperature and the quantity of reproductive organs with viable pollen, pollen germination, pollen tube length and functional activity of the PSA was established (KALAPCHIEVA and PETKOVA, 2004; PETKOVA *et al.*, 2009; NIKOLOVA *et al.*, 2012).

The present investigation is a part of breeding programme for high temperature stress and includes establishing morphological, physiological and productivity response in garden peas during high temperature stress.

MATERIALS AND METHODS

The experiment was carried out in the experimental field of the "Maritsa" Vegetable Crops Research Institute (MVCRI) in Plovdiv, Bulgaria, during the period 2012-2013 by the block method, in four replications with an area of the trial plot 6.4 m^2. The sowing was carried out in the 1st ten-day period of March on a high flatbed by scheme 80 + 20 + 40 + 20 / 4–5 cm (4 rows high flat bed – 160 cm width; the seeds were planted in two couples of double rows 40 cm apart. The distance between the seeds in the row was 4–5 cm and the distance between the rows in the couple was 20 cm. The experiment was irrigated; an irrigated data (irrigation amounts for 2012 are 240 mm and for 2013 – 160 mm) set permits better interpretation of confounding effects of water deficit from temperature effects. Pea was grown under recommended production practices. The fertilization rates were determined in the Agrochemical Laboratory of the Maritsa Vegetable Crop Research Institute, Bulgaria. Fertilizer rates were adjusted according to the soil nutrient status and the biological requirements of garden pea.

For pest and disease control the following plant protection products were applied: Decis 2.5 EC 0.04% (a.i. deltamethrin), Nurele Dursban 0.07% (a.i. cypermetrin + chlorpyrifosetyl) and Mospilan 20 SP 0.0125% (a.i. acetamiprid).

Plant material

Five new Bulgarian lines (72/9, 72/2, 1159, 95/4, 73/10,) and five foreign (Skinado, Jof, Debreceni, Fabundo and Sovin) garden pea varieties of wrinkle seeded type were studied. The breeding material was from the early, mid-early and late groups.

The foreign varieties are a source of initial, genetic material in the breeding together with local varieties and natural populations, so the experiment included varieties from different countries: Fabundo from Germany; Debreceni from Hungary; Sovin from Russia; Jof and Skinado from the Netherlands. Variety Skinado was introduced to our country and it is a standard for peas of the medium-early group.

The studied genotypes of peas were characterized by normal type of leaf with leaflets and tendrils. All ten accessions have seeds from wrinkled type (wrinkling of cotyledon). Three genotypes were with a pointed shape of the distal part of the pod (line 1159, Jof and Debreceni), the other seven were blunt. Maximum number pods of a peduncle (3) were formed by the varieties Skinado and Sovin.

Methods

The vegetation period was recorded /in days/ from emergence to technological maturity when over 50% per plants were growing.

The main morphological and productive characteristics were analyzed on a sample of total 10 plants of the variant in the technological maturity:

Number of nodes up to and including first fertile node - average;

Shape of the distal part of the pod – observation was made on several nodes of each plant when pods are fully developed, but before any senescence;

Length of pod, cm;

Maximum number of pods of peduncle;

Type of leaf - normal with leaflets and tendrils, or afila - only with tendrils;

Number of grains per pod – average;

Seed surface – wrinkled or round;

Seed weight - was measured on at least two samples of 1000 seed. Immature and infected seeds were excluded, g;

Productivity – green pods and green grains yield (kg ha^{-1}).

Weather conditions

An overview of the weather experienced by the varieties in the growing season of each year and the climate normals (1997-2010) are listed in Figures 1. During the vegetative growth period in 2012 year, the mean air temperature was over the normal and in 2013 - around normal. Average precipitation during the growing season was 240 mm in 2012 and was below normal, except at the month May. In second year, precipitations were 303 L.

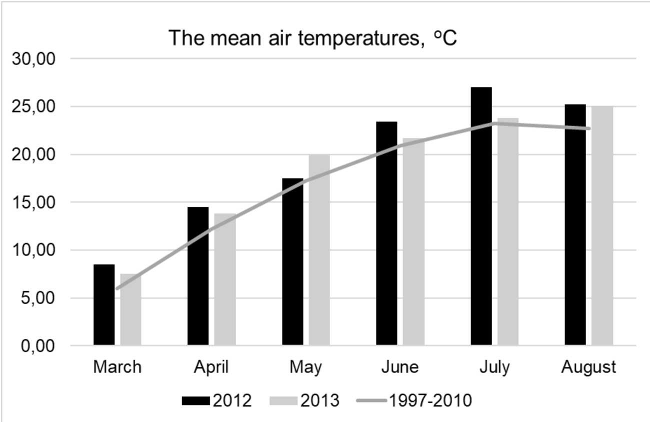


Figure 1. Average monthly air temperatures and precipitation sums for vegetative period (March-August) in 2012 and 2013

Fluorescence measurements

The efficiency of plant photosynthetic apparatus at high temperature was investigated by chlorophyll fluorescence parameters, a modern non-destructive method allowing serial measurements of the same plants in vivo. Parameters of chlorophyll fluorescence at normal temperature (morning measurements) and high temperature (afternoon measurements) were registered three times during the flowering period (30 measurements per accession), which is considered particularly critical for pea plants.

A Portable Fluorimeter PEA (Plant Efficiency Analyzer MK2, Hansatech Instruments Ltd., UK) was used. Fluorescence measurements were taken from intact, fully development dark-adapted (30 min) leaves, on the upper (adaxial) surface illuminated with actinic light (>650nm) with photon flux 1 500 µmol m^{-2} s^{-1}.

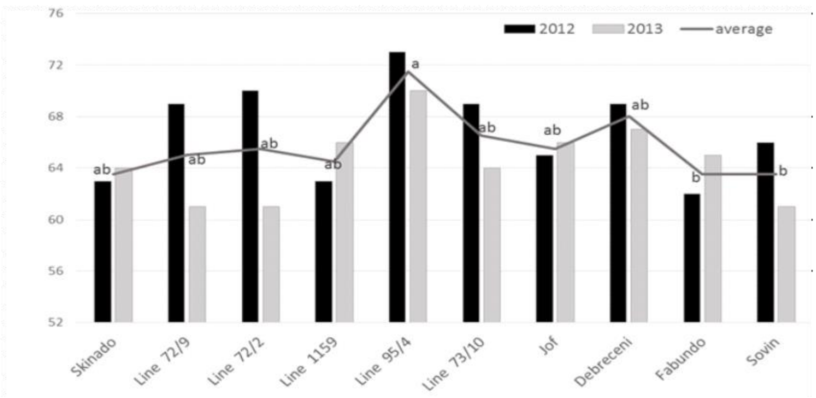
The influence of high temperature on the condition and functional activity of the photosynthetic apparatus (PSA) was studied by analyses of chlorophyll fluorescence characteristics of photosystem II (PSII) in the garden pea leaves – initial fluorescence (F_0), maximum (Fm) and variable (Fv) fluorescence, as well as the ratio Fv/Fm, indicating the potential PS II efficiency in the photochemical reactions.

The initial (minimal) (F_0) fluorescence - represents the fluorescence yield from the reaction center (RC) in the open state; maximum fluorescence (Fm) - represents the maximum fluorescence yield at all closed reaction centers (RC) in which the primary electron acceptor (Qa) is in a reduced state; variable fluorescence (Fv) - describes the change between two defined states of RC ($Fv = Fm-F_0$). They were used to calculate the ratios Fv/F_0 and Fv/Fm, which are considered as indicators for the PS II efficiency in primary photochemical reactions.

The data were statistically processed by the Microsoft Excel software and only differences with **P 0.01 were subject untreated plants.

RESULTS AND DISCUSSION

The differences between accessions of garden pea were significant at the level 0.05 in most measured morphological, productivity characters and vegetation period.



a,b…Duncan,s multiple range test (p<0,05)

Figure 2. Duration of the vegetative period of garden pea lines and varieties, days

The average duration of the vegetation period was varied from 63.5 days for Fabundo to 71.5 days of line 95/4 (Figure 2). Line 95/4 in the both years was characterized with the longest vegetation period - 73 and 70 days, while with the shortest period was variety Fabundo in 2012 and Sovin, lines 72/9 and 72/2 in 2013.

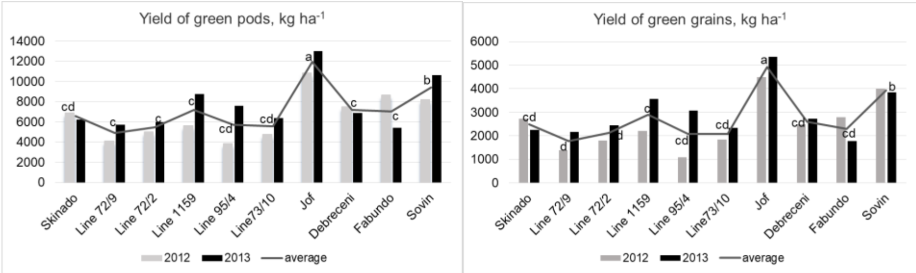
The variety Jof formed first fertile node of the highest located node - 17 in both years, and Line73/10 – on the lowest node – 10 (Table 1). The longest pod (10.7 cm) with the greatest number of grains per pod (7.7) had line 1159 in 2012, while the same line again had the longest pod (9.6 cm) in 2013, but variety Jof had the largest number of grains (7.3). The seed weight was the biggest in Variety Debreceni in 2012 (188 g) and line 1159 in 2013 (223 g), while varieties Fabundo and Skinado produced the lightest seeds i.e., 85 g and 109 g per plant, respectively.

Table.1. Morphological characteristics

Variety, Line	I-st fertile node, number			Length of pod, cm			Number of grains per pod			Weight of 1000 seeds, g		
	2012	2013	average	2012	2013	average	2012	2013	average	2012	2013	average
Skinado	14.0b	12.3g	13.2fg	8.4c	7.5bc	7.9c	6.6b	6.2b	6.4bc	100g	118f	109h
Line 72/9	13.6bc	13.5ef	13.6de	7.5d	6.8c	7.1d	5.3c	5.6b	5.5d	115f	117f	116g
Line 72/2	14.1b	14.2de	14.2d	8.7bc	7.0c	7.9c	5.9b	6.0b	6.0cd	122e	129e	126f
Line 1159	17.0a	15.3cd	16.2b	10.7a	9.6a	10.2a	7.7a	6.5ab	7.1a	175b	223a	199a
Line 95/4	17.0a	14.7d	15.9bc	8.6bc	7.1c	7.9c	6.7b	5.5b	6.1cd	158c	179c	169d
Line 73/10	10.7d	10.9h	10.8g	8.5c	8.1b	8.3c	5.1c	6.2b	5.7d	162c	183bc	173d
Jof	17.4a	17.4a	17.4a	9.4b	9.4a	9.4b	6.6b	7.3a	7.0ab	172b	187b	179c
Debreceni	12.0c	12.7fg	12.5f	7.3de	7.0c	7.2d	3.5d	3.7c	3.6e	188a	186b	187b
Fabundo	13.3bc	17.1ab	15.2c	6.2f	7.1c	6.6e	5.3c	5.8b	5.7d	84h	85g	85i
Sovin	14.4b	16.1bc	15.3c	6.7ef	7.3bc	7.0de	5.9bc	5.6b	5.8cd	133d	170d	151e

a,b…Duncan,s multiple range test (p<0,05)

Genotype Jof was characterized with the largest yield of green pods during both years (10850 kg ha^{-1} for 2012 and 13025 kg ha^{-1} for 2013) and yield of green grains (4492 kg ha^{-1} and 5350 kg ha^{-1}, respectively), (Figure 3).



a,b…Duncan,s multiple range test (p<0,05)

Figure 3. Productivity from green pods and of green grains of garden pea genotypes

The results of this investigation demonstrated that there were no proved differences in the duration of vegetation period of studied genotypes, except of differences between line 95/4 and Fabundo, and Sovin. The studied accessions may be referred to the group of mid-early varieties of a garden pea. The vegetation period at six accessions in the second year was shorter than first year and only varieties Skinado, Jof, Fabundo and line 1159 have duration with 1 to 3 days longer. The differences of vegetation period were strongly related to the common abiotic stress of temperature and water deficit, in addition to other factor such as cultivar and lines originating from different. The pea spent fewer days in reproductive growth when temperatures were greater (BUECKERT *et al*., 2015). The duration from the emergence to the time of flowering phase varies widely across locations and years and the timing of the subsequent stages is closely related to time of flowering (SUMMERFILD and ROBERTS, 1988; TRUONG and DUTHION, 1993). Longer vegetative growth period in 2012 could be due to genetic potentialities of accessions because the combination of higher temperature and precipitation below normal (in March and April) suggested opposite or the difference in precipitation in May of both years when was the period of flowering and the formation of the first pods.

The number of nodes up to and including first fertile node was relatively constant, indirect characteristic for determination of vegetation period length. During the both experimental years the varieties Jof, Debreceni and lines 72/9, 72/9, 73/10 confirmed that. PORYAZOV (1977) reported an increase of node number up to and including first fertile node for mid-early varieties when continuously with the tardiness of sowing which is due to the increase of mean temperature for the period from emergence to flowering In our experiment Skinado, lines 1159 and 95/4 were characterized with bigger number of nodes up to and including first fertile node in 2012, while Fabundo and Sovin - in 2013, when the temperature during the period from emergence to flowering was higher or around average period 1997-2010.

Pod length was measured to identify differences in varieties and lines. Differences among the pea cultivars in terms of pod length were significant ($P < 0.05$).It was determined that there were longer pods in the first year and pod length increased during the second year only varieties Fabundo and Sovin. Weather changes impacted pod length. ESPOSITO *et al*., 2009 concluded that pod length varied with environmental conditions and had increased heritability in a stressful environment compared with non-stressful. We think the genotype also influences.

We observed a no-significant reduction in the number of green grains per pod. Studied genotypes were differentiated in three groups according to the number of grains per pod during first year. In 2013 some accessions increased number of green grains per pod (line 73/10 and Jof), some decreased – lines 1159 and 95/4, others were remained almost unchanged. MAURER *et al.*, (1968), JEUROY *et al.*, (1990) showed that a lack of moisture and high temperatures during flowering reduced the number of seeds per pod. We ascribe these differences to variety response to high temperature.

The higher weight of 1000 seeds during the second year was due to the precipitation (above normal, except at the month May) and temperature - around normal. The heat reduces yield through a combination of fewer reproductive organs carried to maturity and a reduction in seed size (POGGIO *et al.*, 2005).

In our investigation yields of green pods and green grains varied by accessions and year. Generally, hot and dry years correspond to yield reductions. We categorized years 2012 as a hot one based on rainfalls, when the yields in seven accessions were smaller. However, three genotypes were characterized with higher yields of green pods (Skinado, Debreceni, Fabundo) and green grains (Skinado, Fabundo, Sovin) in the same year. The year-to-year variation in yield was most likely influenced by precipitation and temperature, although management practices including irrigation would play a role, as well as genotype. These genotypes could be supposed are more resistant to drought and high temperature conditions.

Seasonal weather reports do not show when extreme weather conditions occur during the growing season, nor do they indicate whether unfavorable climatic conditions coincide with sensitive stages of crop growth such as flowering and the onset of reproductive growth.

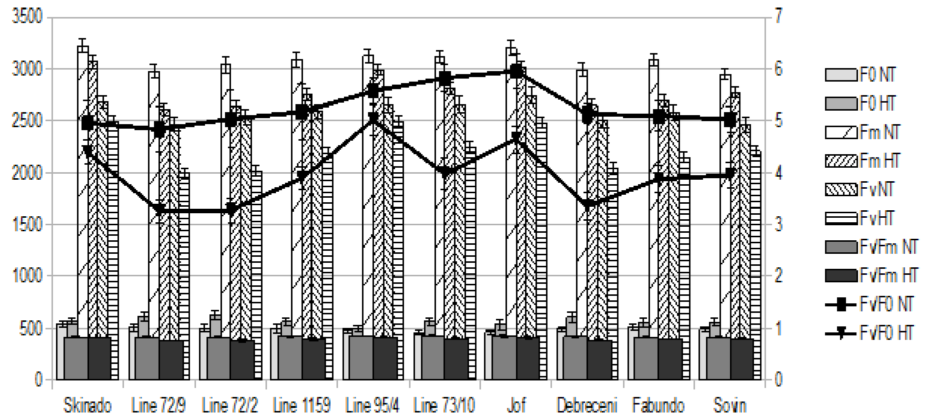


Figure 4. Chlorophyll fluorescence parameters in garden pea accessions during normal (NT), (t_B – 22.4°C) and high temperature (HT), (t_B – 32.8°C), 2012.

Chlorophyll fluorescence characteristics change significantly with thermal damage in photosystem II (PSII) of the photosynthetic apparatus and also impairs electron transfer within the PSII reaction centres (POSPÍSIL and TYYSTJÄRVI, 1999; KOURIL *et al*., 2004; SINSAWAT *et al*., 2004; HALDIMANN and FELLER, 2005). Registered values of chlorophyll fluorescence parameters in morning measurements show a good overall physiological state of the plants of all tested accessions (Figure 4 and 5).

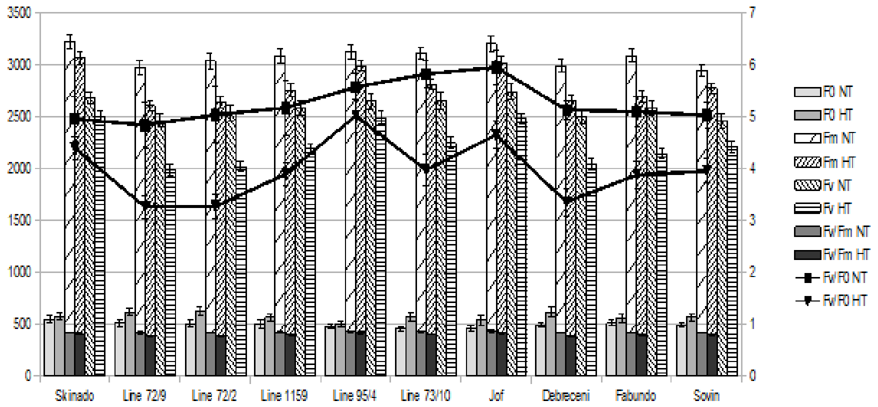


Figure 5. Chlorophyll fluorescence parameters in garden pea accessions during normal (NT), (t°C – 22.0 ± 2°C) and high temperature (HT), (t°C – 33.8 ± 2°C), 2013.

It was found that initial fluorescence F_0 was significantly affected by stress effects from a slight to drastic increase. The increase in F_0 is associated with the release of proteins of the FS2 core complex that bind only a small portion of the chlorophyll molecules. This allows the ratio of F_0 at normal temperature to F_0 at high temperature to be used as a criterion for estimating the stability of the core complex (Table 2). Energy losses increase with increasing temperature. The results obtained in the experimental high temperature conditions show an increase in the values of this parameter in all the studied genotypes (Figure 4 and 5). The increase relative to the control variants varied between 113 (variety Fabundo) - 138% (line 72/2) in 2012 and 105 (Skinado and line 95/4) to 125% for the variety Debreceni in 2013. The initial fluorescence (F_0) describes the loss of the excitation energy during its transfer from the pigment bed to the reaction centre (RC) of PSII. It is reached immediately after illumination of the dark-adapted leaves. In these conditions, most of the RCs of PSII is opened and the first acceptor of the electrons Qa is completely oxidized. We can assume that, the accessions retained the greatest stability of the core complex by the degree of this increase, within the measured temperature values of the order of 33-35ºC.

The variable component of chlorophyll fluorescence (Fv) is a derivative of the initial (F_0) and maximum (Fm) fluorescence. According to some researchers, variable (Fv) fluorescence is a particularly important indicator in the selection of high temperature resistance. The downward tendency in Fv values found in the analysis of the obtained results is due to a decrease in the maximum fluorescence in all the studied accessions. Values of Fv decrease under the influence of high temperature for individual genotypes, reaching maximum of 29% (2012) and 20% (2013) in line 72/2. A significant decrease in this indicator was also found in line 72/9 for the two years, line 1159 in the first year, and the variety Debreceni - the second year. For that reason, plants of same varieties and line 95/4 responded with only a minor reduction of the variable fluorescence at high temperature.

Table 2. Changes in chlorophyll fluorescence parameters (%) in garden peas patterns under high temperature during the period – 2012 – 2013.

Variety	F_0			Fm			Fv			Fv/Fm		
/Line	2012	2013	aver.	2012	2013	aver	2012	2013	aver.	2012	2013	aver.
Skinado	115	105	110.0	95	95	95.0	91	93	92.0	96	98	97.0
Line 72/9	125	120	122.5	85	88	86.5	77	81	79.0	91	92	91.5
Line 72/2	138	124	131.0	81	87	84.0	71	80	75.5	88	92	90.0
Line 1159	120	113	116.5	85	89	87.0	76	85	80.5	90	95	92.5
Line 95/4	114	105	109.5	89	96	92.5	84	94	89.0	95	98	96.5
Line 73/10	114	124	119.0	91	90	90.5	86	85	85.5	95	94	94.5
Jof	114	117	115.5	92	94	93.0	85	91	88.0	92	96	94.0
Debreceni	118	125	121.5	93	89	91.0	88	81	84.5	95	92	93.5
Fabundo	113	109	111.0	100	87	93.5	97	83	90.0	98	95	96.5
Sovin	114	114	114.0	91	94	92.5	87	90	88.5	95	96	95.5

Variety	Fv/F_0			Fm/F_0		
/Line	2012	2013	aver.	2012	2013	aver.
Skinado	79	89	84.0	82	91	86.5
Line 72/9	61	67	64.0	67	73	70.0
Line 72/2	52	65	58.5	59	71	65.0
Line 1159	63	75	69.0	70	79	74.5
Line 95/4	75	90	82.5	78	92	85.0
Line 73/10	76	68	72.0	80	73	76.5
Jof	73	78	75.5	80	81	80.5
Debreceni	75	65	70.0	79	71	75.0
Fabundo	86	76	81.0	88	80	84.0
Sovin	76	79	77.5	80	82	81.0

According to POSPISIL *et al*. (1999), the value of maximum fluorescence (Fm) decreases with the effect of high temperature stresses. In our study, Fm decreased at least in varieties Fabundo and Skinado in both years.

The ratio Fv/F_0 characterized the state and efficiency of the electron transport chain of PSII and the rate of photosynthetic quantum conversion at PSII reaction center (HAVAUX and LANNOYE, 1985; BABANI and LICHTENTHALER, 1996; GAUTAM *et al*., 2014). Decreased values of this fluorescence parameter in afternoon measurements were an indicator of electron transfer disturbance in photosynthetic processes as a result of heat stressed conditions. The depressant influence of high temperature on the electron transport processes of the plants in the experimental conditions was most apparent in accession 72/2 - the average value of ratio Fv/Fo was 65% relative to the control (Table 2). This indicator is least affected by the high temperature in the varieties Line 95/4 and Skinado.

The ratio variable/maximum fluorescence (Fv/Fm) was a parameter characterizing the potential PSII efficiency in the photochemical reactions. It reflected the thermo-induced changes in the overall quantum efficiency of PSII and under stress effects, its values decrease. The results obtained for the Fv/Fm under normal temperature (morning measurements) conditions confirmed the range established by BOLHÀR-NORDENKAMPF and ÖQUIST (1993) from 0.75 to 0.82 (Figure 4 and 5). Therefore, under normal conditions, plants did not significantly differ by the potential of photochemical conversion of light energy.

In afternoon measurements under the influence of high temperature, the Fv/Fm values decreased for all studied genotypes (Figure 4 and 5). Our data show that among the studied genotypes, the ratio Fv/Fm was more reduced under applied stress for lines 1159, 72/2 in 2012 and 72/2, 72/9, Debreceni in 2013. Line 72/2 also showed a more reduction of the PSII efficiency (<10% to the control) in both years, (Table 2). In variety Jof the value of the ratio Fv/Fm was below the physiological norm (0.693) in 2012 (Figure 4). The variety Fabundo in 2012 and line 95/4 in 2013 was characterized with the highest potential of quantum yield.

Changes in morphological characteristics, productivity and fluorescence emission parameters in garden pea, depending on the accessions and conditions of the years have been identified. Varieties of Skinado and Fabundo respond with higher yields of green pods and grains in the conditions of the first year, categorized as hot compared to 2013. The screening for thermostability of the photosynthetic apparatus to the high temperature characterizes Skinado, Fabundo and line 95/4 as the most tolerant on the basis of the analysis of fluorescence induction parameters, and the most sensitive – lines 72/2 and 72/9. We consider that the indicated accessions are suitable for the pea-breeding programme to high temperature stress tolerance.

Received,February 15th, 2018
Accepted May 18th, 2019

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MORFOLOŠKI, FIZIOLOŠKI I PRODUKTIVNI ODGOVOR U GENOTIPOVIMA GRAŠKA PRI STRESU VISOKE TEMPERATURE

Slavka KALAPCHIEVA*, Elena TOPALOVA, Valentina PETKOVA

Departman za oplemenjivanje, Održavanje i introdukcija sorata, Maritsa istraživački institut za povrtarstvo, Plovdiv, Bugarska

Izvod

Utvrđene su promene morfoloških karakteristika, produktivnosti i parametara fluorescentne emisije pri normalnim i visokim temperaturama u poljskim uslovima kod 10 sorti i linija vrste *Pisum sativum* L. iz kolekcije MARITSA Instituta za povrtarstvo. Uticaj visoke temperature na stanje i funkcionalnu aktivnost fotosintetskog aparata (PSA) ispitivan je analizom fluorescentnih karakteristika hlorofila fotosistema II (PSII) u lišću graška. Biljke su bile izložene stresu visoke temperature tokom reproduktivnog perioda (stvaranje pupoljka - faza cvetanja), što se smatra posebno kritičnim za biljke graška. Na morfološke karakteristike i produktivnost uticale su klimatske karakteristike u ispitivanim godinama. Skrining za termostabilnost fotosintetskog aparata na visoku temperaturu karakterisao je Skinado, Faboundo i liniju 95/4 kao najtolerantnije na osnovu analize parametara indukcije fluorescencije. Najosetljivije linije bile su 72/2 i 72/9. Sorte Skinado i Fabundo reagovale su višim prinosima zelenih mahuna i zrna u uslovima prve godine, kategorisane kao veoma tople u odnosu na 2013. Smatramo da su navedeni uzorci iz kolekcije pogodni za program oplemenjivanja graška na tolerancije na stress visoke temperature.

Primljeno 15.II.2018.
Odobreno 18. V. 2019.