ВЛИЯНИЕ НА ВИСОКАТА ТЕМПЕРАТУРА ВЪРХУ ЖИЗНЕНОСТТА НА ПОЛЕНА ПРИ ОБРАЗЦИ ОТ *PISUM SATIVUM* L.
HIGH TEMPERATURE INFLUENCE ON THE POLLEN VIABILITY OF *PISUM SATIVUM* L. ACCESSIONS

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Резюме

Изследвано е влиянието на високата температура (ht) върху жизнеността на полена при 23 образци от *P. sativum* от ранната, средно ранната и късната група от колекциите на Института по зеленчукови култури "Марица" (ИЗК "Марица") и Института по растителни генетични ресурси (ИРГР). В предишно наше проучване с три моделни сорта грах беше установено, че температурните режими 45°C/2 и 3 h (t стойност и продължителност на въздействие), които са с близка до полулеталната t за жизнеността на полена, са подходящи за подбор на толерантни към ht стрес генотипи. В това проучване, използвайки посочените температурни режими, беше установена отрицателна зависимост между продължителността на третиране с ht и количеството на репродуктивни органи с жизнен полен, прорастването на полена (%) и дължината на поленовата тръбичка (μm). От колекцията на ИЗК „Марица" сортът Мусала (ранна група) показа висок процент на репродуктивни органи с поленова жизненост (70 и 44% - при третиране 2 и 3 h, съответно). Сортът Пулпудева и линия 2213 от същата група показаха подобен отговор към t режим 45°C/2 h. Сортът Хемус (средно ранна група) и сортът Успех (късна група) показаха толерантен към ht мъжки гаметофит. При образците от ИРГР беше установена генотипна диференциация при третиране с 45°C/2 h. Като най-перспективни се откроиха сортовете Froidure и Picardi и линиите 470-26, A6BM008 и A6BM0011.

Abstract

The influence of high temperature on the pollen viability in 23 *P. sativum* accessions, of early, mid-early and late groups from the collections of the *Maritsa* Vegetable Crops Research Institute, Plovdiv (MVCRI), and the Institute of Plant Genetic Resources *K. Malkov* (IPGR), Sadovo, was studied.

In our preliminary study with three model pea cultivars it was found that the temperature regimes 45°S / 2 and 3 hours, close to the semi-lethal temperature for pollen viability, are suitable for selection of genotypes tolerant to high temperature. In this study, using the above temperature regimes, a negative relation was found between the treatment continuance and quantity of the reproductive organs with viable pollen, pollen germination (%), and pollen tube length (μm). Among the studied accessions from MVCRI the cv. *Musala* (early group) maintained high quantity of the reproductive organs (70 and 44% - 2h and 3h treatments) with different pollen viability. The cv. *Pulpudeva* and line 2213 from the same group showed comparable response to the temperature regime 45°C/2h. The cv. *Hemus* (mid-early group) and cv. *Uspech* (late group) manifested a male gametophyte tolerant to high temperature. A genotypic differentiation in the treatment at 45°C/2h was established in the IPGR accession group. Cv. *Froidure* and *Picardi* and lines 470-26, A6BM008 and A6BM0011 had the highest potential for high temperature tolerance.

Ключови думи: *Pisum sativum*, високотемпературен стрес, жизненост на полена.
Key words: *Pisum sativum*, heat stress, pollen viability.

INTRODUCTION

High temperature stress above 30°C during bud formation and blossoming periods of pea species leads to an abortion and falling off of the reproductive organs (Guilion et al., 1997, 2003) and to decrease of the photosynthetic activity (Georgieva and Lichtenthaler, 1999; Petkova et al., 2008). A breeding programme for abiotic stress tolerance in *Pisum sativum* species was developed at MVCRI (Kalapchieva and Petkova, 2004; Nikolova et al., 2008; Tomlekova, 2010). The male gametophyte tolerance to high temperature has been investigated in pot and laboratory experiments. Initially, critical temperature value of 45°C for 2 and 3 h for pollen viability was established in three pea model cultivars (Nikolova et al., 2008; Petkova et al., 2008, 2009). The present study is a second part of our programme aimed at screening tolerant to high temperature stress pea genotypes.

MATERIAL AND METHODS

The studies were conducted from 2003 to 2007. Pea plants were grown in plastic 5 L pots containing commercial soil-peat substrate in 3 plants per pot. Four pots per treatment were used for each accession. The plants were treated during the blossoming period with high temperature at 45°C for 2 and 3 hours. Untreated plants were used as control.

Plant materials

The following pea accessions were included in the study:

From the MVCRI gene pool:

- Early group – cultivars Pulpudeva, Musala, Ateroy and line 2213;
- Mid-early group – cultivars Hebar, Plovdiv, Hemus and Konkord;
- Late group – cultivars Uspech, Viatovo, Plovdiv pearl and line 97/3.

From the IPGR gene pool:

- Cultivars Exclusive, Froidure, Picardi and Amitie
- Lines № 470-17, 470-24, 470-26, A6BM008, A6BM009, A6BM0011 and №11.

Cytological investigation

The pollen viability in buds with size from 0.8 to 1.5 cm and in flowers of the treated plants was determined by the hanging drop method using two characteristics – pollen grain germination (%) and pollen tube elongation (*l*, μm). The pollen was sown in Petri dishes with nutritive medium containing agar, saccharose, H_3BO_3 and $CaCl_2$. The percentage of germination and the pollen tube lengths wåre determined 24 hours after placing the Petri dishes in a thermostat at 26°C. The same parameters were measured in buds and flowers of the control untreated plants.

RESULTS AND DISCUSSION

The study established a negative effect of high temperature stress on pollen germination and pollen tube elongation in all studied cultivars and lines from the MVCRI collection.

Early group

The pollen kept viability of 79.2% and 69.6% of the reproductive organs of cultivars Pulpudeva and Musala at 45°C for 2h (Fig. 1). In vitro pollen grains germination on the nutritive medium averaged 40.6% and 24.9%, respectively in these two species (Table 1). The temperature regime we applied had a lethal effect ($\bar{x}$ = 0) on the male gametophyte in 44.5% of the buds and flowers from line 2213. The reproductive organs of these accessions drooped and fell off down after the treatment. Cultivar Ateroy showed the highest pollen sensitiveness among the studied genotypes.

It was found that the negative effect of high temperature on male gametophyte becomes stronger with prolongation of the treatment by an hour (3 h). In cultivar Pulpudeva and line 2213, 91.7% and 82.1% of the buds and flowers, respectively, were without any pollen viability (= 0) (Fig.1).

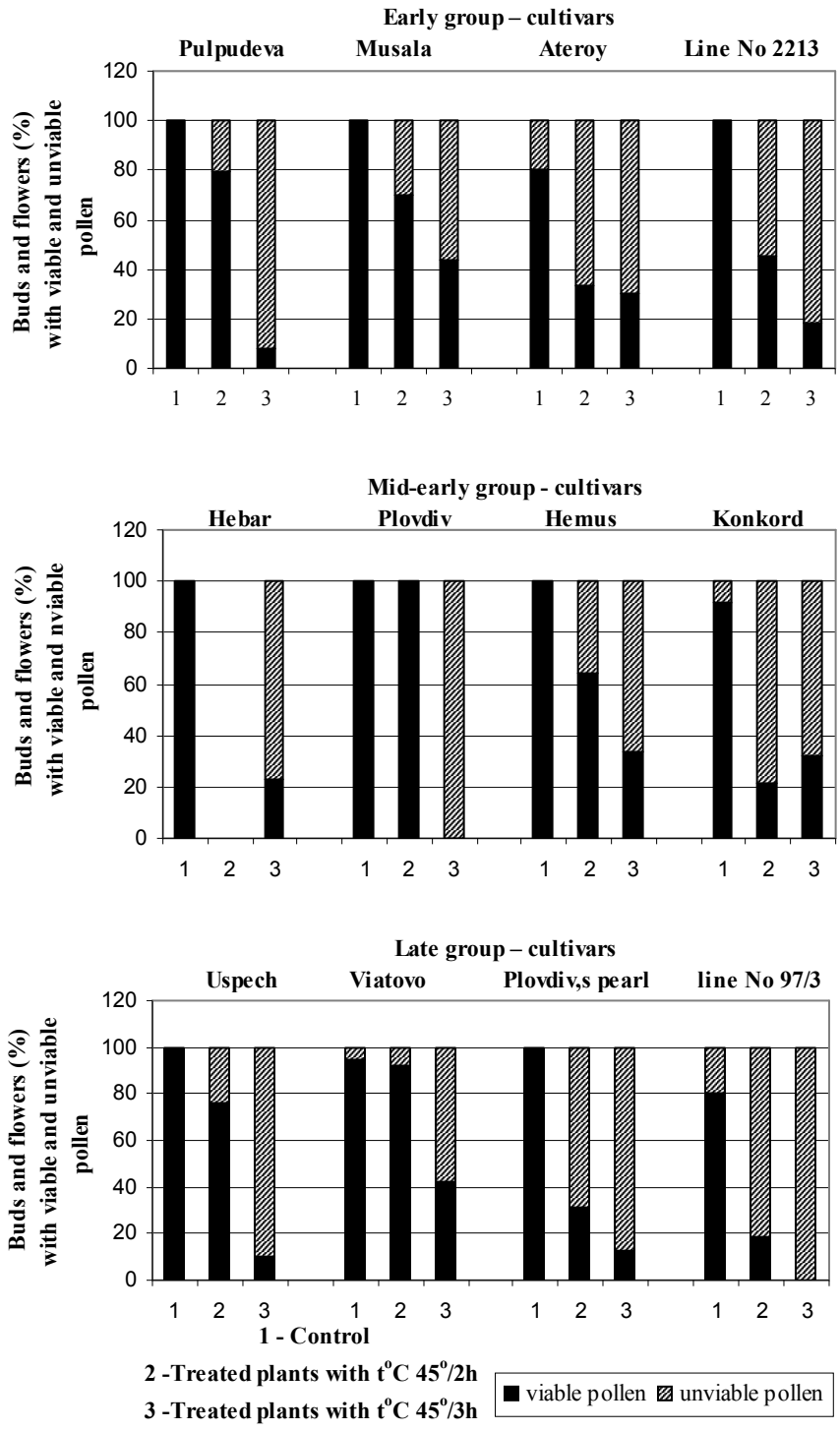
In the remaining reproductive organs 3.8% and 10.5% of the pollen grains and the pollen tube reaches 121.1 and 305.6 μm length, respectively. The reaction of the male gametophyte of cultivar Ateroy did not change considerably under the influence of the temperature regime with longer temperature duration (45°C for 3h), compared to the influence of the temperature 45°C for 2h. Significant differences in the percentage of the reproductive organs with the degenerated pollen and in the values of pollen germination and pollen tube elongation were not found in this cultivar.

The obtained data from the treatment with two combinations of high temperature and continuation manifested a different reaction of the male gametophytes of the studied accessions. Plants of the cultivar Ateroy expressed a specific response to the applied two temperature regimes. This cultivar showed high sensitiveness in treatment with 45°C for 2h in comparison to the other genotypes, however using the second temperature regime (45°C for 3h) in a few buds and flowers it germinated high quantity of the pollen grains (x_{max} = 45.3%) with good elongation of the pollen tubes (l_{max} = 1023.9 μm).

Mid-early group

Cultivars Plovdiv and Hemus demonstrated certain tolerance at temperature treatment at 45°C for 2h. The pollen kept its viability in 100% and 64.5% of the studied generative organs (Fig. 1) - the values of $\bar{x}$ and *l* were 40.9 and 43.3% and 743, 6 and 725, 0 μm, respectively. The negative effect of the applied regime was strongest in the cultivar Konkord.

The second temperature regime a t 45°C for 3 hours was lethal for the male gametophyte of cultivar Plovdiv. The establishment of certain tolerance in



Фиг. 1. *Бутони и цветове (%) с жизнен и нежизнен полен в контролни и третирани с висока температура растения на грахови образци (колекция ИЗК „Марица”)*

Fig. 1. *Buds and flowers (%) with viable and unviable pollen in control and in high temperature treated plants of the pea accessions (collection MVCRI)*

Таблица 1. Влияние на високата температура върху жизнеността на полена при образци от *Pisum sativum* (колекция на ИЗК „Марица")

Table 1. High temperature influence on the pollen viability of *Pisum sativum* accessions (MVCRI collection)

Accessions Образци	t regimes t режими	Pollen germination Прорастване на полена ($\bar{x}$, %)			Pollen tube length Дължина на поленовата тръбичка (ℓ, μm)		
		x_{min} (%)	x_{max} (%)	$\bar{x} \pm c$ (%)	ℓ_{min} (μm)	ℓ_{max} (μm)	$\ell \pm c$ (μm)
Early group / Ранна група							
Cv.Pulpudeva Пулпудева	Control	60.6	100.0	95.0 ± 8.6	458.6	1447.2	985.9 ± 217.8
	t° C 45°/2h	1.1	100.0	40.6 ± 40.4	206.7	1050.5	591.2 ± 281.8
	t° C 45°/3h	1.4	6.1	3.8 ± 3.3	84.4	157.8	121.1 ± 51.9
Cv. Musala Мусала	Control	4.3	100.0	90.4 ± 21.4	391.3	1704.9	1010.8 ± 338.1
	t° C 45°/2h	2.0	96.7	24.9 ± 26.8	128.1	1180.9	650.2 ± 380.1
	t° C 45°/3h	1.5	19.7	9.4 ± 6.6	73.4	749.8	421.6 ± 241.4
Cv. Ateroy Атерой	Control	3.3	97.5	56.9 ± 40.6	240.1	1797.9	1073.1 ± 452.7
	t° C 45°/2h	3.8	37.3	13.3 ± 13.8	344.5	1279.3	651.2 ± 334.4
	t° C 45°/3h	0.6	45.3	14.5 ± 17.3	335.1	1023.9	656.6 ± 268.7
Line 2213 Линия 2213	Control	30.3	100.0	84.8 ± 19.9	554.5	1327.7	1094.9 ± 178.4
	t° C 45°/2h	0.5	79.2	40.9 ± 27.7	108.6	948.2	491.3 ± 272.6
	t° C 45°/3h	1.9	23.5	10.5 ± 10.6	152.1	408.1	305.6 ± 102.8
Mid-early group / Средно ранна група							
Cv. Hebar Хебър	Control	80.6	100.0	94.8 ± 6.6	237.4	1053.8	712.2 ± 256.5
	t° C 45°/2h	-	-	-	-	-	-
	t° C 45°/3h	0.3	6.6	2.9 ± 2.8	90.6	398.3	227.4 ± 121.7
Cv. Plovdiv Пловдив	Control	41.5	100.0	82.4 ± 17.3	721.6	1429.2	1176.6 ± 175.6
	t° C 45°/2h	2.4	95.0	40.9 ± 32.8	304.6	1187.7	743.6 ± 299.7
	t° C 45°/3h	0.0	0.0	0.0	0.0	0.0	0.0
Cv. Hemus Хемус	Control	2.5	95.0	56.2 ± 32.3	92.2	1767.4	1090.3 ± 564.8
	t° C 45°/2h	2.4	95.0	43.3 ± 39.0	171.8	1396.5	725.0 ± 429.2
	t° C 45°/3h	1.1	29.5	8.0 ± 8.8	132.8	768.2	252.6 ± 196.2
Cv. Konkord Конкорд	Control	1.3	95.0	45.6 ± 39.2	195.3	1757.2	849.9 ± 677.5
	t° C 45°/2h	3.3	9.3	5.8 ± 2.1	162.5	205.5	174.2 ± 16.0
	t° C 45°/3h	0.4	7.4	3.4 ± 2.6	125.8	238.3	176.2 ± 36.2
Late group / Късна група							
Cv. Uspech Успех	Control	9.2	95.0	63.1 ± 36.4	193.7	1441.0	989.4 ± 397.8
	t° C 45°/2h	2.3	100.0	62.1 ± 34.6	601.4	1498.0	1075.3 ± 289.3
	t° C 45°/3h	1.6	4.9	3.3 ± 2.3	231.7	247.3	239.5 ± 11.0
Cv. Viatovo Вятово	Control	46.8	100.0	84.5 ± 14.4	511.6	1466.0	1050.5 ± 251.1
	t° C 45°/2h	2.0	90.0	31.1 ± 23.8	171.1	821.7	302.4 ± 188.6
	t° C 45°/3h	6.0	63.4	22.8 ± 23.3	182.0	587.7	275.1 ± 142.5
Cv. Plovdiv Pearl/пловд. Перла	Control	5.0	100.0	68.9 ± 32.6	301.5	998.1	569.2 ± 164.1
	t° C 45°/2h	0.8	40.3	9.9 ± 11.3	117.2	317.1	214.1 ± 61.4
	t° C 45°/3h	1.3	3.8	2.2 ± 1.1	130.2	312.4	227.8 ± 74.8
Line 97/3 Линия 97/3	Control	75.4	90.0	85.6 ± 6.9	343.7	1128.6	875.2 ± 363.6
	t° C 45°/2h	4.2	92.6	48.4 ± 62.5	242.1	246.3	244.2 ± 3.0
	t° C 45°/3h	0.0	0.0	0.0	0.0	0.0	0.0

temperature treatment for 2 hours and the presence of lethality in prolongation of the duration by 1 hour, demonstrates a specific reaction of the male gametophyte of this cultivar to the applied temperature influence. The temperature treatment for 3 hours of the other three cultivars reduced considerably: (i) the quantity of the reproductive organs in which the pollen has kept its viability (from 23.1% to 33.3%); (ii) the pollen germination (from 2.9% to 8.0%) and (iii) the pollen tube length (from 176.2% to 252.6 μm).

Late group

Certain tolerance of the male gametophyte was established in the 2 hour treatment of the cultivars Viatovo and Uspeh. The pollen kept its viability in 91.7% and 76.2% of the reproductive organs (Fig. 1), at an average of 31.3% and 62.1%, respectively, of the pollen grains germinated. The pollen germination in untreated control plants of cultivar Uspeh (63.1%) did not differ significantly from the established value in the two hour treatment (62.1%). In this cultivar the high temperature slightly stimulated the elongation of the pollen tubes (*l*= 1075.3 m). Longer duration of the temperature influence has the lowest expressed negative effect on the male gametophyte in cultivar Viatovo – on average 22.8% of the pollen sown on nutritive media germinated in 42.1% of the studied buds and flowers. We observed that the genotype determined the specific response to the applied abiotic stress also in the late group accessions. For example, the high temperature stress in both durations of the treatment of cultivar Viatovo damaged to a great degree the elongation of the pollen tubes in a considerable part of the germinated pollen grains.

Table 2 illustrates that the cultivars Picardi, Froidure and lines 470-26, A6BM008, and A6BM 0011 have relatively tolerant response. The male gametophyte kept its viability to varying degrees (from 11.5% to 67.2%) in a large percentage of the buds and flowers (from 84.6% to 100%) under temperature treatment of the plants at 45°C for 2h. The tolerance of these accessions was confirmed from the established maximum values of germination (x_{max} from 41.4 to 92.0%) in individual flowers of some plants. High values of the x_{max} confirmed the possibility for choice of tolerant genotypes on the basis of the response of the male gametophyte to temperature stress at 45°C for 2h. Prolongation of the treatment (3 hours) in considerably: most of the studied accessions did not increase considerably the percentage of the buds and flowers with zero value of the pollen viability, however it decreased the values of %, as the germinated pollen did not exceed 10.0% in eight of the studied eleven cultivars and lines (Table 2). The negative effect of the temperature at 45°C for 3 hours was the slightest in lines A6BM008 and A6BM0011.

The plants from lines 11 and A6BM009 were the ones most susceptible to the temperature stress, in which part of the buds and flowers died in two prolongations of

Таблица 2. Влияние на високата температура върху жизнеността на полена при образци от *Pisum sativum* (колекция на ИРГР)

Table 2. High temperature influence on the pollen viability of *Pisum sativum* accessions (IPGR collection)

Accessions Образци	Buds and flowers Бутони и цветове: with unviable pollen с нежизнен полен (%)			Buds and flowers Бутони и цветове: with viable pollen с жизнен полен (%)			Pollen germination Прорастване на полена $\bar{x} \pm c$ (%)			Pollen tube length Дължина на поленовата тръбичка $\ell \pm c$ (μm)		
	Control Контрола	t regime t режим 45°C/2h	t regime t режим 45°C/3h	Control	t regime t режим 45°C/2h	t regime t режим 45°C/3h	Control Контрола	t regime t режим 45°C/2h	t regime t режим 45°C/3h	Control Контрола	t regime t режим 45°C/2h	t regime t режим 45°C/3h
Exclusive	17.6	50.0	31.2	82.4	50.0	68.8	32.1 ± 25.6	2.6 ± 1.8	1.5 ± 0.8	411.6 ± 269.9	128.2 ± 25.2	137.1 ± 32.2
Froidure	6.1	8.0	50.0	93.9	92.0	50.0	25.2 ± 18.7	11.5 ± 10.7	3.6 ± 3.3	444.5 ± 242.2	432.9 ± 227.9	210.0 ± 138.8
Picardi	30.0	15.4	59.1	70.0	84.6	40.9	66.1 ± 29.9	17.0 ± 16.8	1.4 ± 0.8	481.6 ± 389.9	221.3 ± 102.4	212.8 ± 102.7
Ametie	0.0	-	50.0	100.0	-	50.0	59.2 ± 0.0	-	1.2 ± 0.7	794.2 ± 0.0	-	177.9 ± 69.2
L. 11	0.0	66.7	64.3	100.0	33.3	35.7	33.4 ± 20.4	1.8 ± 1.1	1.1 ± 0.6	616.3 ± 218.4	172.3 ± 32.1	131.1 ± 30.0
L.470-17	0.0	12.5	16.7	100.0	87.5	83.3	61.8 ± 0.0	14.4 ± 21.1	5.8 ± 6.5	589.9 ± 0.0	298.5 ± 397.5	158.7 ± 34.9
L.470-24	6.7	35.3	19.0	93.3	64.7	81.0	61.0 ± 27.6	2.7 ± 2.4	14.1 ± 15.8	661.7 ± 391.4	247.7 ± 123.4	453.6 ± 325.6
L.470-26	0.0	0.0	25.0	100.0	100.0	75.0	26.5 ± 25.9	23.1 ± 20.4	4.7 ± 8.5	551.3 ± 309.4	390.5 ± 282.0	300.1 ± 216.6
L. A6BM008	0.0	7.1	5.9	100.0	92.9	94.1	59.5 ± 31.4	54.0 ± 28.2	11.9 ± 11.8	780.3 ± 405.2	664.7 ± 264.1	392.1 ± 131.6
L. A6BM009	0.0	57.1	50.0	100.0	42.9	50.0	29.0 ± 31.8	2.6 ± 2.6	17.4 ± 0.0	386.3 ± 351.9	160.6 ± 42.5	348.8 ± 0.0
L. A6BM0011	28.6	11.5	0.0	71.4	88.5	100.0	67.2 ± 22.0	22.8 ± 22.7	9.5 ± 6.6	786.7 ± 492.8	298.5 ± 247.1	514.2 ± 332.2

the treatment, as their pollen was completely unviable and it could not germinate on the stigmas. Analysis of the second characteristic of the pollen viability, established that the length reached by the pollen tubes in treated plants was slightly reduced in the first temperature regime compared to the temperature impact for 3 hours.

CONCLUSION

The temperature regimes, including the value of 45°C and continuance of 2 and 3 hours, are suitable to test the MVCRI and IPGR pea collections and to select genotypes tolerant to high temperature stress.

The study established a negative relationship between the duration of the treatment (2 and 3 hours) and the quantity of reproductive organs with viable pollen, pollen germination and pollen tube length. A genotypic differentiation and the specific reaction of the different male gametophytes in treatment with applied temperature regimes allow the selection of genotypes tolerant to high temperature stress.

The cultivars Pulpudeva and Musala belonging to the early pea group, Hemus from the mid-early group and Uspeh and Viatovo from the late group of the MVCRI collection possess good tolerance of male gametophyte to the high temperature stress.

Cultivars Froidure and Picardi and lines 470-26, A6BM008, and A6BM0011 have the highest potential for high temperature tolerance from the IPGR, Sadovo accession's group.

After the test of the pea collections, the accessions will be classified by heat stability of their male gametophyte and the best ones will be used in the breeding process of *Pisum sativum* species.

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