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Heat Tolerance and Adaptability to Drought in Sunflower Can Be Influenced by Pollen Selection

Abstract: The heat tolerance and adaptability to drought of F_2 sporophytic populations after F_1 sunflower pollen heating have been studied. Freshly collected pollen was heated at the temperature of 60°C during 1 and 3 h and used to self-pollinate the emasculated F_1 plants. Half of F_2 seeds were treated with the temperature of 60°C during 15 min before sowing while another was not subjected to high temperature. The number of F_2 flowering plants during 2013 dry summer season was counted. Pollen treatments compared with the control (fresh pollen) significantly increased the number of F_2 plants after seed heating before sowing. In some F_1 hybrids, pollen heating during 1 h was not effective. Pollination of F_1 hybrids with pollen treated with the temperature of 60°C during 1 h compared with fresh pollen increased the number of F_2 plants in dry field conditions. Obtained results show that pollen selection was successful to increase the heat tolerance and adaptability to drought in sunflower.

Keywords: Helianthus, F_1 hybrids, pollen selection, F_2 sporophytic generation, heat tolerance, drought tolerance

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Introduction

Cultivated sunflower is the main oil crop in Ukraine. The crop faces with drought and high temperature in the south regions of this country those are suitable for the growing of sunflower. This crop encounters with similar problems in other countries too.

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Drought is one of the most complex and destructive abiotic stressor in global scale. Drought damage exceeds the damage from any other stresses. During vegetative development, drought reduced the main stem height, stem diameter, number of nodes or leaves, and leaf area. The number of leaves and root weight are the best selection criteria to determine drought tolerance at early vegetative stage (Onemli and Gucer, 2010). It should be noted that drought is usually accompanied by temperature increase which invokes damage of the plants and decreases the yield. Increasing the drought and heat tolerance of the plants are the important directions of the modern plant breeding and plant cultivation.

Development of modern and high-yielding varieties and hybrids of the plants that can resist to different stress factors carries out usually at the adult vegetating plant stage using traditional methods. Selection of drought and heat resistant plants can be implemented at different stages of the sporophytic development. However, it is possible to realize the selection at the gametophytic stage. It is usually carried out by using the microgametophyte. In the natural conditions, pollen selection causes the adaptation of the progeny to the ecological niche. Internal gametophytic selection has already occurred during the pollen maturation. External gametophytic selection takes place during transfer of the microgametes, its germination, and growth of the pollen tubes (Zhuchenko, 2001).

It was established that a large part of the structural genes expressing in the pollen also exhibits in sporophyte. This fact indicates that gene transcription occurs in the haploid genome and therefore can take place the selection of the traits, which are controlled by genes expressing at both sporophyte and gametophyte stages. Success of the microgametophytic selection depends also on the presence of genetically various pollen (Mulcahy, 1979).

Advantages of the gametophytic selection are the microscopic size and haploid genotype of the pollen that ensures an expression of the recessive genes, which determine the agriculturally important traits of the cultivated plants. Involvement in the artificial selection of a large number of the genotypes and possibility to regulate the selection force under the strictly controlled conditions are also indisputable advantages of the pollen selection (Ottaviano and Mulcahy, 1989).

Several directions of the pollen selection application had already established. They are microgametophytic ecotypical breeding for tolerance to abiotic factors and phytopathogens; using populations of pollen grains as the test system to study the plant adaptation to ecological niche; and treatment of pollen grains by the DNA fragments to broaden the selection pool under interspecific hybridization. Pollen selection technique can also be used for the meiosis correction, using pollen treatment with BAS, to save the valuable recombinant gametes at the postmeiosis stages of combinational breeding (Pivovarov *et al.*, 2003).

Spectrum of the crops that show possibility of the selection of valuable genotypes at the microgametophytic stage is sufficiently wide and includes the important species such as tomato, cucumber, maize, cabbage, cotton, rape, flax, sunflower, and others. List of the abiotic and biotic environmental factors for carrying out the successful microgametophytic (pollen) selection is large enough too. They are low and high temperatures, chlorine and sulfate salinization, heavy metals, herbicides, and different diseases (Zamir *et al.*, 1982; Sacher *et al.*, 1983; Searcy and Mulcahy, 1985; Rodriguez-Garay and Barrow, 1988; Sari Gorla *et al.*, 1989; Hodgkin, 1990; Lyakh *et al.*, 2000). On the whole, using the pollen selection method the effectiveness of the breeding process can be significantly improved. At the same time, the combined selection (selection at the sporophyte and gametophyte stages) is believed to be more effective (Kilchevsky and Pugacheva, 2002).

The aim of the present paper was to study the influence of pollen heating in F_1 sunflower hybrids on the F_2 resulting sporophytic offspring for the traits of heat tolerance and adaptability to drought.

Materials and methods

F_1 hybrids "*virescent*" × "*xantha*", "dichotomous venation" × "burnt leaf" and "dichotomous venation" × "*xantha*" were taken as the experimental material. The parental lines of these hybrids were obtained through induced mutagenesis and characterized by essential differences for heat and drought tolerance (Soroka and Lyakh, 2009; Lyakh *et al.*, 2009).

F_1 hybrids were grown in the field conditions during 2012. Before pollination, the inflorescences of F_1 plants of each crossing combination were isolated. One set of these plants was emasculated within 1–5 days for artificial pollination. The other set was grown without emasculation for collecting fresh pollen. Freshly collected pollen was immediately transferred (in 5–10 min after collection) to the laboratory, was placed in parchment packets in a layer of 2–3 mm height and heated at the temperature of 60°2°C using air bath oven for a period of 1 and 3 h for "dichotomous venation" × "burnt leaf" and "dichotomous venation" × "*xantha*" at 60 ± 2°C for a period of 1 h for "*virescent*" × "*xantha*" and then used to self-pollinate the emasculated F_1 plants. Viability test based on pollen germination on artificial nutrient medium (Lyakh *et al.*, 2000) showed that about 1–3% of pollen grains were viable after heating for a period of 3 h. Freshly collected pollen without temperature treatment was used for control pollinations. One cm^3 of pollen was taken for pollination of each head.

The seeds were sown in early May 2013. Half of the seeds both control and experimental heads were treated with the temperature of 60°C for a period of 15 min before sowing (Polevoy *et al.*, 2001). The other half were not subjected to high temperature. Influence of F_1 pollen heating on the heat tolerance of F_2 segregating populations was estimated by the counting the number of flowering plants during 2013 summer season after seed treatment. Effect of pollen selection for adaptability to drought was determined by comparing the number of flowering plants in the F_2 populations obtained after pollen heating and without pollen treatment in dry conditions of 2013.

In 2013, rainfall in May was 29 mm and 31.5 mm in June, while the average long-term rates were 40 mm and 62 mm, respectively. This means that the amount of rainfall during the 2 months of vegetation before flowering was only about 60% of the long-term average (Table 1). Before seeding in April and early May, there was almost no rainfall, while the temperature was significantly higher than the average long-term rates.

Table 1: Weather conditions before and after sowing of sunflower, 2013

Decade	**Month**					
	April		**May**		**June**	
	Average daily temperature, t°C	**Rainfall, mm**	**Average daily temperature, t°C**	**Rainfall, mm**	**Average daily temperature, t°C**	**Rainfall, mm**
I	10.2	0	22.9	0	22.3	21.5
II	12.6	8.0	22.3	25.0	25.0	10.0
III	16.9	0	24.0	4.0	26.7	0
Average temperature	13.2	–	23.1	–	24.7	–
Total rainfall	–	8.0	–	29.0	–	31.5
Average long-term rates	8.5	35	16.0	40	19.4	62

The differences between control and experimental variants (treatments) as well as differences among the treatments were defined by the t-test at the levels of probability of 0.01 and 0.001.

Results and discussion

Data on the influence of pollen heating in F_1 hybrids on the heat tolerance of F_2 resulting sporophytic offspring are presented in Table 2. Before sowing, the F_2 seeds were heated to estimate the heat tolerance of the analyzed material. The number of flowering F_2 plants developed without pollen treatment and obtained using the pollen selection technique was used for comparison of the heat tolerance in experimental and control populations.

Table 2: Influence of pollen heating in F_1 hybrids on heat resistance of F_2 resulting sporophytic offspring

Treatment	Number of F_2 seeds treated with high temperature	Number of F_2 flowering plants	Frequency of F_2 plants, %
F_2 *virescent* × *xantha*			
Control	414	15	3.6 ± 0.92
60°C/1 h	144	42	29.2 ± 3.79***
F_2 dichotomous venation × burnt leaf			
Control	884	31	3.5 ± 0.62
60°C/1 h	912	43	4.7 ± 0.70
60°C/3 h	605	113	18.7 ± 1.59***,###
F_2 dichotomous venation × *xantha*			
Control	864	56	6.5 ± 0.84
60°C/1 h	827	243	29.4 ± 1.58***
60°C/3 h	543	128	23.6 ± 1.82***

Notes: ***The differences from the control are significant at the 0.001 level of probability; ###the differences between treatments are significant at the 0.001 level of probability.

Table 2 shows that number of flowering plants in F_2 populations obtained by pollen treatment for a period of 1 h at the temperature of 60°C was significantly higher than the number of plants in the control in the "*virescent*" × "*xantha*" and "dichotomous venation" × "*xantha*" cross combinations. This indicates the increase of the frequency of the heat tolerant plants in F_2 populations under pollen treatment. The same differences were not significant in the "dichotomous venation" × "burnt leaf" cross combination. Apparently, at the given temperature pollen treatment was insufficiently hard to select the heat tolerant genotypes.

The number of F_2 plants developed after pollen heating for a period of 3 h was significantly higher than the number of control F_2 plants in the "dichotomous venation" × "burnt leaf" and "dichotomous venation" × "*xantha*" cross combinations. These results allow make the same conclusion that we made after comparison of control F_2 populations and the populations developed with using the 1-h gametophytic selection.

In "dichotomous venation" × "burnt leaf" cross combination, the number of F_2 plants obtained by means of the 3 h gametophytic selection exceeded the number of plants developed under 1 h pollen heating. However, the significant difference in the number of the F_2 plants between control and experimental F_2 populations was not observed in the "dichotomous venation" × "*xantha*" cross combination.

Data on the influence of pollen heating in F_1 hybrids on the adaptability to drought in F_2 resulting sporophytic offspring are presented in Table 3. To estimate the effectiveness of pollen treatment, the comparison between the number of flowering plants obtained without pollen treatment in F_1 and the number of flowering plants developed under pollen treatment was carried out. For cultivated sunflower, the environmental field conditions during 2013 season were arid, at the initial stages of growth and development especially.

Table 3: Influence of pollen heating in F_1 hybrids on adaptability to drought in F_2 resulting sporophytic offspring

Treatment	Number of F_2 seeds	Number of F_2 flowering plants	Frequency of F_2 plants, %
F_2 *virescent* × *xantha*			
Control	168	75	44.6 ± 3.84
60°C/1 h	105	55	52.4 ± 4.87
F_2 dichotomous venation × burnt leaf			
Control	137	81	59.1 ± 4.20
60°C/1 h	540	395	73.1 ± 1.91**
60°C/3 h	154	103	66.9 ± 3.79
F_2 dichotomous venation × *xantha*			
Control	250	167	66.8 ± 2.98
60°C/1 h	514	469	91.2 ± 1.25***
60°C/3 h	18	14	77.8 ± 9.80

Notes: **, ***the differences from the control are significant at the 0.01 and 0.001 levels of probability, respectively.

The number of flowering plants was significantly higher in F_2 populations obtained at 1-h pollen treatment than in the control in "dichotomous venation" × "burnt leaf" and "dichotomous venation" × "*xantha*" cross combinations. In other cases under temperature pollen treatment the tendency to increasing the number of flowering plants in F_2 populations compared with the control was observed. These facts indicate that gametophytic selection for heat tolerance increases the adaptability to drought of the F_2 populations.

Significant decrease of the number of flowering F_2 plants developed after heating F_2 seeds (Table 2) in comparison of plants obtained without seed treatment (Table 3) was found in all cross combinations. This is the result of the death of the insufficiently heat resistant genotypes after temperature treatment of the seeds as well as the low frequency of the heat resistant plants in F_2 population.

In our earlier studies, we tested the selective effect of different temperatures on the heterogeneous pollen populations of interspecific sunflower hybrids *H. annuus* × *H. argophyllus* and *H. annuus* × *H. bolandery* (Lyakh *et al.*, 1998). The results showed that pollen heating at 40°C for 3 h did not cause the selective elimination of haploid genotypes. Only the use of a higher temperature for pollen treatment led to modifying the genetic structure of sporophytic population. Thus, pollen heating at a temperature of 60°C for 3 h compared to 1 h was more effective.

It was reported earlier that pollen selection was successful for increasing the resistance of plants to different abiotic stress factors in different crops. Pollen selection for heat resistance was successful in tomato (Kravchenko *et al.*, 1988). According to the authors, the heat resistance of the varietal and segregating populations obtained with heated-up pollen exceeded the heat resistance of the control populations for 3–10%. The effectiveness of pollen selection for heat tolerance was shown in maize (Lyakh and Soroka, 1993).

Pollen selection technique for heat and drought tolerance has been successfully implemented in the breeding of a number of crops. For example, when using the treatment of a heterogeneous pollen population with the temperature of 40°C for 30 min the linseed variety “Pivdenna nich” was created. It proved highly adaptive to arid conditions of southern Ukraine and for more than 10 years is the national standard (Lyakh and Soroka, 2008). Cold resistant “Pamyati Zhigalova” variety of the sweet pepper was obtained using both sporophytic and gametophytic selection (Pivovarov, 2008). There is also information that in Japanese turnip “Snegurochka” variety was created by pollen selection (Pivovarov *et al.*, 2003).

Thus, the heat tolerance and adaptability to drought in sunflower can be influenced by pollen selection. However, for the most effective selection for heat resistance the individual force of selection should be chosen in each genotype. Increase of the heat and drought tolerance of F_2 populations developed by using the gametophytic selection proves need of its implementation in sunflower breeding for resistance to unfavorable factors that allow to increase the frequency of valuable genotypes in the populations.

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RESISTENCIA AL CALOR Y LA CAPACIDAD DE ADAPTACIÓN A LA SEQUÍA EN GIRASOL PUEDE SER INFLUENCIADA POR LA SELECCIÓN DE POLEN

RESUMEN

La resistencia al calor y la capacidad de adaptación a la sequía de las poblaciones esporofíticas F_2 después de calentar el polen de los hibridos F_1 de girasol han sido estudiados. Polen recogido recién se calentó a la temperatura de 60°C durante 1 y 3 horas y se utiliza para auto-polinizar las plantas F_1 castrados. La mitad de las semillas F_2 se trató con la temperatura de 60°C durante 15 min antes de la siembra, mientras que otro no se sometió a alta temperatura. Se contó el número de plantas F_2 con flores durante 2013 estación seca del verano. Tratamientos de polen en comparación con el control (polen fresco) aumentó significativamente el número de plantas F_2 después de calentar las semillas antes de la siembra. En cierto híbridos F_1 el calentamiento de polen durante 1 hora no era eficaz. La polinización de híbridos F_1 con polen tratados con la temperatura de 60°C durante 1 hora en comparación con polen fresco aumentó el número de plantas F_2 en condiciones de campo seco. Los resultados obtenidos muestran que la selección de polen fue exitosa para aumentar la resistencia al calor y capacidad de adaptación a la sequía en girasol.

RÉSISTANCE À LA CHALEUR ET ADAPTATION DE SECHERESSE DANS LE TOURNESOL PEUT ETRE INFLUENCÉE PAR POLLEN SÉLECTION

RESUME

La résistance à la chaleur et de l'adaptabilité à la sécheresse des populations sporophytiques F_2 de tournesol après le chauffage de pollen d'hybrides F_1 ont

été étudiés. Pollen fraîchement prélevé a été chauffé à la température de 60°C pendant 1 à 3 heures et utilisé pour l'auto-pollinisation des plantes F_1 émasculés. La moitié des graines F_2 a été traité avec la température de 60°C pendant 15 min avant de les semer, tandis qu'un autre n'a pas été soumis à une température élevée. Le nombre de plantes à fleurs F_2 en 2013 la saison sèche de l'été a été prise en compte. Traitements de pollen par rapport à la commande (pollen frais) ont augmenté de manière significative le nombre de plantes F_2 après le chauffage des graines avant de les semer. Dans un certain hybrides F_1 chauffage de pollen pendant 1 heure n'a pas été efficace. La pollinisation des hybrides F_1 avec pollen traités avec la température de 60°C pendant 1 heure par rapport à pollen frais a augmenté le nombre de plantes F_2 dans les conditions de terrain sec. Les résultats obtenus montrent que la sélection de pollen a réussi à augmenter la résistance à la chaleur et de l'adaptabilité à la sécheresse chez le tournesol.