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Selective Elimination of Gametes during Pollen Storage at Low Temperature as a Way to Improve the Genetic Structure of Sporophytic Population for Cold Tolerance

Abstract: The genetic structure of F_2 sporophytic populations after F_1 sunflower pollen storage at low temperature has been studied. Freshly collected pollen was stored at the temperature of 3 ± 1°C for a period of 7–8 days and used to self-pollinate the emasculated F_1 plants. F_2 seeds were germinated at 5 ± 1°C, and then the percentage of seed germination was counted. Germinated and not germinated seeds were separately planted in the phytotron at an optimum temperature. Segregation ratios in F_2 populations for marker traits were analyzed at the stage of the second pair of true leaves. Pollen treatment compared with the control (fresh pollen) significantly changed in F_2 populations monogenic ratios for some marker traits. In some cross combinations, increase in the cold tolerance of F_2 populations was found. Obtained results show that pollen storage at low temperature selectively influences the male gametophytes of F_1 hybrids that change the genetic structure of F_2 populations.

Keywords: sunflower, F_1 hybrids, pollen storage, low temperature, selective elimination, F_2 sporophytic generation

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Introduction

It is known that many genes are expressed at the level of gametophyte at different stages of its development, including pollen maturation, mature pollen

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grain, pollen germination and tube growth. Many of these genes are expressed both at the gametophytic and at the sporophytic levels (Mulcahy, 1979; Hormaza and Herrero, 1992). This is the basis for the selective elimination of gametes, which may lead to a change in the structure of segregating sporophytic populations.

One of the first who found the selective elimination of gametes was Brink (1925). He stored F_1 maize pollen over calcium chloride at 40°C and revealed the change in Mendelian segregations in F_2 populations in the direction of increasing the number of plants with waxy seeds.

Subsequently, there was a lot of work concerning the selective influence of various agents on the pollen of different plant species. It was shown that maize pollen storage for a long time not only at low but also at room temperature could change the structure of sporophytic populations due to selective elimination of male gametes. In this case, both the changes in monogenic ratios for some marker genes and in evaluation of recombination frequency between them were observed (Lyakh and Soroka, 1992). Heather J. Clarke *et al.* revealed the change in F_2 population structure for flower color after growing of F_1 chickpea hybrid plants at low temperature (Clarke *et al.*, 2004). Significantly modified monogenic and digenic ratios for two DNA markers were found after pollen selection for resistance to toxins of wilt pathogen in *Cicer arietinum* L. (Ravikumar *et al.*, 2006).

It is now known that pollen selection for tolerance to many abiotic factors, including low temperature, is quite effective. Mature pollen storage at low temperature increased the cold tolerance in tomato (Kravchenko *et al.*, 1988), rape, flax (Lyakh *et al.*, 2000) and other crops. Pollen selection for cold tolerance during pollen germination and tube growth has been successfully used in tomato (Zamir *et al.*, 1982; Dominguez *et al.*, 2005) and chickpea (Heather J. Clarke *et al.*, 2004). There were also positive results on the macrogametophytic (ovule) selection for cold tolerance in tomato (Kravchenko *et al.*, 1988).

Acreage expansion of sunflower to the north is limited by unsufficient cold tolerance of plant, especially at early stages. For a successful early sowing of sunflower, it is important to increase the cold tolerance during seed germination, at the seedling and 2–3 pairs of leaves stages. To cultivate sunflower at high altitudes and in cold regions, frost tolerance during the plant ripening should be increased. Some wild species of *Helianthus*, growing in cold conditions, could serve as the sources of frost tolerance. However, sunflower breeding for cold tolerance was not almost conducted (Skoric, 2009). At the same time, some cold tolerant varieties of sunflower using cold seed germination test have been revealed (Sirotin *et al.*, 2007).

The aim of this paper was to investigate the influence of F_1 pollen storage at low temperature on the genetical structure of F_2 segregating

populations including the monogenic ratios for some marker traits and cold tolerance of sporophytic generation.

Materials and methods

F_1 sunflower hybrids of "dichotomous venation" × "*xantha*", "*xantha*" × "dichotomous venation", "*xantha*" × "dwarf" cross combinations were used as the material for research. The parental lines of these hybrids were contrasting in cold tolerance.

"*Xantha*", "dichotomous venation" and "dwarf" lines were obtained through experimental mutagenesis. "Dichotomous venation" mutant sample has the marker trait of modified leaf venation. In contrast to the original line, which has reticulate venation, the mutant is characterized by a dense network of the fan-shaped veins. "Dwarf" mutant has shorter internodes, compact habit, serrate leaf margin and possesses the xeromorphic traits. Both mutant traits are easily identified at early stages of plant development (Lyakh *et al.*, 2005).

F_1 hybrids were grown in the field conditions during 2013. Pollen mixture of several F_1 plants was placed in parchment packages (1 cm^3 per package) and stored in a refrigerator at 3 ± 1°C for a period of 7 days for "dichotomous venation" × "*xantha*" and "*xantha*" × "dwarf" plants and 8 days for "*xantha*" × "dichotomous venation" cross combinations. Viability test showed that pollen treatment significantly decreased pollen germination on the artificial nutrient medium. After that previously emasculated F_1 plants of the same cross combination were pollinated with stored pollen. F_1 plants pollinated with fresh pollen were used as the control.

Cold resistance of F_2 sporophytic populations was evaluated by the seed germination at low temperature. For this purpose seeds were treated with 1% $KMnO_4$ solution for a period of 10 min. The seeds were then placed in Petri dishes on a filter paper previously moistened with distillated water. It was boiled beforehand for a period of 5 min, and then nystatin (250 thousand units/L) and Previkur (2 mL/L) were added. Closed Petri dishes were placed in a refrigerator at 5 ± 1°C. After 7 days, the percentage of seed germination was calculated (Polevoy *et al.*, 2001).

Germinated and not germinated seeds were separately planted in wooden boxes in the phytotron at an optimum temperature. The genetic structure of F_2 segregating populations for "dichotomous venation" and "dwarfness" marker traits was analyzed at the stage of the second pair of true leaves. The following comparisons were performed: (a) experimental (stored pollen) to control (fresh

pollen) F_2 populations, composed of seeds germinated and not germinated in Petri dishes at low temperature; (b) experimental (stored pollen) to control (fresh pollen) F_2 populations, composed of seeds germinated in Petri dishes at low temperature; (c) F_2 population, composed of seeds germinated in Petri dishes at low temperature, to F_2 population, composed of seeds not germinated in Petri dishes at low temperature, both experimental and control.

The differences in cold tolerance between the control and the experimental populations were defined by the *t*-test at the levels of probability of 0.001. Differences in the segregation ratio were evaluated using the χ^2 method.

Results and discussion

As is shown in Table 1, pollen storage at low temperature in F_1 sunflower hybrids changed the genetic structure of F_2 populations for "dichotomous venation" and "dwarfness" marker traits.

Table 1: Influence of low temperature pollen storage in F_1 sunflower hybrids on segregation ratio in F_2 generation for "dichotomous venation" and "dwarfness" marker traits

Pollen storage time	F_2 phenotypes		Segregation ratio	χ^2
	Normal plants	Plants with marker trait		
Dichotomous venation × *xantha*				
Fresh pollen (control)	179	50	3.6:1	20.6
7 days	244	113	2.2:1**	
Xantha × dichotomous venation				
Fresh pollen (control)	167	53	3.2:1	0.9
7 days	155	41	3.8:1	
Xantha × dwarf				
Fresh pollen (control)	176	58	3:1	4.0
7 days	39	22	1.8:1*	

Notes: $\chi^2_{0.05}(df = 1) = 3.84$. * and ** are significant at the 0.05 and 0.001 levels of probability, respectively.

As compared to the control low temperature storage of heterogeneous F_1 pollen population significantly increased in F_2 sporophytic populations the number of plants with "dichotomous venation" and "dwarfness" marker traits in

"dichotomous venation" × "*xantha*" and "*xantha*" × "dwarf" cross combinations, respectively. Thus, we can say that such procedure favors gametes with the named marker traits. As a result, in F_2 populations the number of plants possessing these marker traits was increased. However, the change of the genetic structure of F_2 population was not observed in "*xantha*" × "dichotomous venation" cross combination.

Segregation ratios for marker traits in F_2 populations, composed only of seeds which germinated in Petri dishes at low temperature, were analyzed in Table 2. This part of F_2 population is the most cold tolerant part.

Table 2: Genetic structure of F_2 sunflower populations composed of germinated at low temperature seeds

Pollen storage time	F_2 phenotypes		Segregation ratio	χ^2
	Normal plants	Plants with marker trait		
Dichotomous venation × *xantha*				
Fresh pollen (control)	161	41	3.9:1	27.0
7 days	232	108	2.1:1**	
Xantha × dichotomous venation				
Fresh pollen (control)	62	24	2.6:1	0.9
8 days	112	36	3.1:1	
Xantha × dwarf				
Fresh pollen (control)	125	32	3.9:1	4.0
7 days	26	13	2:1*	

Notes: $\chi^2_{0.05}(df = 1) = 3.84$. * and ** are significant at the 0.05 and 0.001 levels of probability, respectively.

The data presented in Table 2 pointed out that low temperature pollen storage in F_1 hybrids of "dichotomous venation" × "*xantha*" and "*xantha*" × "dwarf" cross combinations increased the number of plants with marker traits "dichotomous venation" and "dwarfness" in the most cold tolerant parts of F_2 populations, respectively. This effect was not observed in "*xantha*" × "dichotomous venation" cross combination.

Table 3 shows the comparison of the genetic structure of F_2 populations, composed of seeds that were germinated and not germinated at low temperature in Petri dishes, both experimental and control. This will allow to evaluate the influence of pollen treatment on the difference in segregation ratios of analyzed phenotypes between control and experimental F_2 populations.

Table 3: Phenotypic ratios in F_2 sunflower populations composed of germinated and not germinated at low temperature seeds

Pollen storage time	F_2 seeds after germination at low temperature	F_2 phenotypes		Segregation ratio	χ^2
		Normal plants	Plants with marker trait		
Dichotomous venation × *xantha*					
Fresh pollen	Germinated	161	41	3.9:1	2.8
(control)	Not germinated	18	9	2:1	
7 days	Germinated	232	108	2.1:1	0.1
	Not germinated	12	5	2.4:1	
Xantha × dichotomous venation					
Fresh pollen	Germinated	62	24	2.6:1	2.5
(control)	Not germinated	105	29	3.6:1	
8 days	Germinated	112	36	3.1:1	5.1
	Not germinated	43	5	8.6:1*	
Xantha × dwarf					
Fresh pollen	Germinated	125	32	3.9:1	8.5
(control)	Not germinated	51	26	2:1**	
7 days	Germinated	26	13	2:1	0.6
	Not germinated	13	9	1.4:1	

Notes: $\chi^2_{0.05}(df = 1) = 3.84$. * and ** are significant at the 0.05 and 0.01 levels of probability, respectively.

In the control, the difference in segregation ratios between F_2 population, composed of germinated seeds, and F_2 population, composed of not germinated seeds, was not observed in "*xantha*" × "dichotomous venation" crossing combination. In the experimental F_2 population however, such difference was evident. The thing was that the storage at low temperature of heterogeneous pollen population of this F_1 hybrid increased the number of plants with the "dichotomous venation" marker trait in F_2 population, composed of germinated at low temperature seeds, compared with F_2 population, composed of not germinated at low temperature seeds. This indicates that treatment of pollen with low temperature increases in F_2 population the proportion of cold tolerant genotypes possessing the "dichotomous venation" marker trait. Despite the fact that the effect of pollen treatment on the genetic structure of F_2 population according to the data in Tables 1 and 2 was not observed, such effect was found in this comparison.

A similar situation of the selective elimination of gametes after pollen treatment was observed in "*xantha*" × "dwarf" cross combination. The

difference in the segregation ratios between F_2 population, composed of germinated at low temperature seeds, and F_2 population, composed of not germinated at low temperature seeds, was revealed in the control in the direction of increasing the number of plants of the "dwarf"-type among the not germinated seeds. However, no difference in segregation ratios was found in the experimental population. Thus pollen treatment, as compared to the control, increased the number of plants with the "dwarfness" marker trait in F_2 population, composed of germinated at low temperature seeds. The data of segregation ratios for both cross combinations show the change in F_2 population structure after low temperature storage of pollen in F_1 hybrids.

Summarizing the data presented in Tables 1–3, it is possible to draw a general conclusion that pollen storage at low temperature influences selectively the male gametophytes of F_1 hybrids that changes the genetic structure of F_2 populations. This selective influence resulted in the increase in F_2 the number of genotypes with "dichotomous venation" and "dwarfness" marker traits.

The change in the cold tolerance of F_2 populations also indicates the changes in the genetic structure of these populations after pollen treatment in F_1 hybrids (Table 4). This cold tolerance was determined by the percentage of seed germination at low temperature.

Table 4: Influence of pollen storage at low temperature in F_1 hybrids on cold tolerance of F_2 populations in sunflower

Pollen storage time	F_2 seeds		Germination, %
	Total	Germinated	
Dichotomous venation × *xantha*			
Fresh pollen (control)	365	269	73.7 ± 2.30
7 days	415	364	87.7 ± 1.61*
Xantha × dichotomous venation			
Fresh pollen (control)	543	114	21.0 ± 1.75
7 days	276	160	58.0 ± 2.97*
Xantha × dwarf			
Fresh pollen (control)	471	189	40.1 ± 2.26
7 days	138	46	33.3 ± 4.01

Notes: *differences are significant at the 0.001 level of probability.

Pollen treatment in "dichotomous venation" × "*xantha*" and "*xantha*" × "dichotomous venation" cross combinations increased the percentage of F_2 seeds germinated at low temperature from 73.7% to 87.7% and from 21.0% to

58.0%, respectively. In "*xantha*" × "dwarf" cross combination, the percentage of F_2 seed germination at low temperature in the experiment did not differ from the control. In this case, pollen storage was not effective to enhance the cold tolerance of F_2 population.

Taking into account that pollen treatment in F_1 hybrids of "dichotomous venation" × "*xantha*" and "*xantha*" × "dichotomous venation" cross combinations increases the cold tolerance of F_2 populations and at the same time the number of plants with "dichotomous venation" marker trait we can assume that the gene, which determines this marker trait, is at least partially linked to the loci(locus) that determine(s) the cold tolerance in sunflower.

The effect of F_1 hybrids pollen storage at low temperature on the structure of F_2 sporophyte populations was earlier studied in sunflower. It was found that such pollen treatment increased the number of plants with the traits of more cold resistant parent (Gasenko and Lyakh, 1997). However, those data did not allow to conclude about the change in the cold tolerance of the experimental population after pollen selection application.

In sunflower, the selective elimination of the gametes was also observed after heating heterogeneous pollen population in F_1 hybrids. Such treatment favored the male gametes possessing the genes which determine heat and drought tolerance (Lyakh and Totsky, 2014).

The obtained results, indicating that during pollen storage at low temperature the selective elimination of gametes is observed, should be taken into account in sunflower breeding programs as many valuable genotypes can be lost due to such procedure.

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