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POLLEN SELECTION TECHNIQUES FOR OILSEED CROP BREEDING

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The results for the development of methods of microgametophytic selection in oilseed crops are presented. It is shown the possibility of oil-bearing crops pollen to preserve the fertilizing ability under different temperature conditions. The heating of a heterogeneous pollen population increases the drought resistance of F_2 sporophytic populations in linseed and the heat and drought resistance of the resulting offspring in sunflower. The possibility of selecting cold-resistant genotypes during the period of pollen germination and pollen tube growth was revealed in spring rape. The effectiveness of technology on microgametophytic selection of castor-bean genotypes which are tolerant to *Fusarium* wilt is shown. When the most competitive gametes were involved in fertilization, the frequency of early flowering genotypes in the sporophytic population have substantially increased in linseed and castor-bean. The developed methods allow to accelerate the process of creating the valuable breeding material in oilseed crops.

Key words: microgametophytic selection, pollen, oilseed crop, abiotic factor, biotic factor.

Introduction. One of the limiting factors to create high-yielding varieties and hybrids of cultivated plants is a lack of simple but at the same time reliable methods for evaluation and selection of valuable genotypes at the early stages of the breeding process. Selection at the level of microgametophyte could play an important role in solving this issue. The principle possibility for such selection is based on the expression of a significant part of the sporophyte genome during the haploid phase of plant development [1].

Male gametophyte (pollen) has a number of features that allow it to be successfully used in breeding programs. One of the main characteristics of a male gametophyte is its small size, which varies from 2 μm in the Forget-me-not to 200 μm in the pumpkin plants. The microscopic size of the pollen makes it possible to analyze a large number of genotypes. In addition to the small size, the pollen is characterized by a haploid state of the genome, which, unlike the diploid state, allows one to detect both rare recessive alleles and adaptive characteristics controlled by a large number of loci. These features constitute a significant advantage of gametophyte selection over the traditional methods [1, 2].

Along with developing methods for microgametophyte selection, it is equally important to develop methods for evaluating the quality of sporophyte by its male gametophyte. The fundamental possibility for creating such methodical approaches is also based on the expression of a significant part of the sporophyte genome genes in the gametophyte. A possibility to use them at the earliest stages of the breeding process, especially when the breeding material is quantitatively limited, is the advantage of the proposed assessment methods. Evaluation at the level of pollen may also be of independent interest, since the sensitivity to a particular factor of the plant reproductive system itself is a very important characteristic for many agricultural crops [3].

Experimental studies performed with different crops in 1980-1990 have shown that gametophytic selection is effective for such traits as resistance to high and low temperatures, salinity in tomato, high temperature in cotton, high and low temperatures, herbicides in maize, and others [4-6]. The almost complete absence of information on the issue under consideration for oilseed crops, served as an impetus for the development of microgametophytic selection methods in rapeseed, flax, sunflower, castor-bean, which are important agricultural objects for many countries.

Material and methods. The techniques for most of the manipulations with pollen of oil-bearing crops presented in the current article are described in more details in the methodical recommendations [7]. To study the duration of preservation of the fertilizing ability of the flax pollen, it was stored at the temperature of 18 ± 1 °C and 3 ± 1 °C for 10 days. The fertilizing ability was judged by the percentage of seeded bolls and their insemination.

The temperature background for selection in F_1 generation of cold-resistant genotypes of spring rape during the period of pollen germination and pollen tube growth was that of 3-9 °C for 2 days after transferring pollen onto the stigmas. The efficiency of selection was judged by the percentage of F_2 seeds germinated under the cold conditions.

Pollen selection for cold tolerance in sunflower was carried out by keeping the pollen of hybrid plants at the temperature of 10 and 3 °C for 10 days. Seeds resulted after pollination with fresh and stored pollen were sown in a field to evaluate the structure of F_2 populations in plant height and duration of "seed emergence – flowering" period.

Selection of drought-tolerant flax genotypes at the stage of mature pollen grains was carried out by heating the F_1 hybrid pollen at 35 °C for 1-2 hours. After germination of BC_1 seeds on a medium with osmotic, the percentage of seed germination and the length of the embryonic roots were determined.

To assess the effect of selection on the heat resistance of sporophyte populations in sunflower, its pollen was heated at the temperature of 60 ± 2 °C for 1 or 3 hours. Heat resistance was estimated by the ability of seeds to germinate after their incubation in a water bath at 60 °C for 15 min [8].

Pollen selection for resistance of castor-bean to Fusarium wilt was performed by applying pollen on the stigma, moistened previously with a 10% culture filtrate of the *Fusarium oxysporum* pathogen [9].

To ensure pollination with a limited amount of pollen, a stigma of a spring rapeseed flower, emasculated a day before flowering, was loaded with 5-50 pollen grains using a tip of a dissection needle.

Results and discussion. Ability of pollen to preserve for a long time its vitality and fertility is an important condition for successful manipulation of the male gametophyte. Therefore, we have studied the possibility of pollen to preserve the above-mentioned characteristics under different temperature conditions for a number of oil-bearing crops. In particular, the fertilizing ability of pollen was studied in oil flax when pollen was stored in room conditions and at a lower temperature (Table 1).

The studies have shown that pollination, executed with pollen which was stored even for a short period (3 days) at the temperature of 18 ± 1 ° C, led to a significant decrease in both the boll set and seed set. Storage of pollen at this temperature for a longer period caused a complete loss of its fertilizing ability. At the same time, the temperature regime of 3 ± 1 °C made it possible to maintain a sufficiently high

fertilizing ability of pollen within 10 days [7]. The identified so-called "time intervals of life" for the pollen allowed further conscious manipulation with the male gametophyte in the experiments on pollen selection.

Table 1

Fertilizing capacity of oil flax pollen depending on the duration of its storage at different temperatures
(1997-1999)

Pollen storage time	Fruit set, %		Seed set, pcs.	
	temperature, °C		temperature, °C	
	18±1	3±1	18±1	3±1
	Antares × (Tsian × Antares)			
Control (fresh pollen)	100.0	100.0	8.2	8.2
3 days	5.1*	66.7*	3.0*	7.6
7 days	0.0	69.2*	0.0	8.0
10 days	0.0	53.3*	0.0	6.4*

* – differences from the control are significant at the 1% level of significance

Based on the fact that a significant proportion of genes expressed in pollen are also expressed in sporophyte, one could expect that the selection in a heterogeneous population of microgametophytes for tolerance to a factor, for example, to cold or high temperature, should provide an increase in resistance to the same factor of the resulted generation of sporophytes. Studies carried out with various oilseed crops have confirmed this assumption.

F.e., the possibility of selecting cold-resistant genotypes during the period of pollen germination and pollen tube growth was studied in spring rape [7]. Hybrid plants, generated after crossing contrasting for cold resistance samples, after pollination with their own pollen were immediately transferred to a background of low temperatures 3-9 °C. After 2 days the plants were placed under optimum conditions, previously removing the stigma and the upper part of the style to prevent the germination of pollen that did not germinate in the cold. In the control, the germination of pollen and the growth of pollen tubes proceeded at the optimum temperature. The resulted F_2 seeds were germinated in the cold as presented in table 2.

As can be seen from the table, the experimental populations significantly exceeded the control population by the ability of the seeds to germinate under conditions of low temperature (germination temperature of 2 °C). In the control on the 3rd and 5th days of cold germination seeds failed to germinate, on the 10th day they just started germination, and on the 12th day the amount of germinating seeds in the control was lower than in the experimental populations by 3-5 times.

Thus, by selecting more cold-resistant microgametophytes at the stage of pollen germination and pollen tube growth, cold resistance of the resulted sporophyte generation was changed. Those temperature regimes of pollen treatment formed the basis of the developed technique for selection of cold-resistant genotypes in rape.

Table 2

The effect of low temperature during pollen germination and pollen tube growth of the F_1 rape hybrid Dneprovsky × 0438 on the cold tolerance (seed germination, %) of the F_2 sporophyte generation

(1997-2000)

Temperature during germination of pollen on the stigma	Time of seed germination, days			
	3	5	10	12
Control, 25°C	0	0	0.4±0.38	7.5±1.56
Treatment 1, 3°C	0	0	1.0±1.03	37.5±5.02*
Treatment 2, 9°C	6.7±1.62*	8.0±1.76*	8.0±1.76*	27.0±2.88*

* – differences from the control are significant for $p \leq 0.001$.

Cold tolerance of sporophytes can also be enhanced by pollination of the pollen that has been stored for a certain time at low temperature. As an example we can cite the data obtained on sunflower [10]. F_1 hybrid Leader × Skorospely was used as the material. Parental components of that hybrid differed significantly both in cold resistance and in a number of other quantitative characteristics, such as the duration of the "seed emergence - flowering" period and plant height: Leader variety – cold-resistant, tall, late-flowering; Skorospely variety – non-cold-resistant, short, with a short "seed emergence – flowering" period. Before pollination pollen of the hybrid was stored in a refrigerator at the temperature of 3 and 10 °C for 7 and 10 days. Pollination in control was carried out with freshly collected pollen. Seeds resulted after self-pollination were sown in the soil to analyze the plants for duration of the "seed emergence - flowering" period and the height (Table 3).

Table 3

Influence of cold treatment of F_1 sunflower pollen on the plant segregation in F_2 populations for some quantitative traits, %

(1999-2000)

Pollen treatment	Plant height, cm			"Seedling emergence – flowering" period	
	up to 150	151-175	>175	up to 60 days	>60 days
Control	19.4	64.1	16.5	79.3	20.7
3°C, 10 days	12.4	59.6	28.0*	4.4***	95.6***
10°C, 10 days	9.5	49.4***	41.1***	54.2***	45.8***

*, *** – differences from control are significant for $p \leq 0.05$ and 0.001, respectively.

Analysis of the structure of F_2 populations showed that storage of the F_1 hybrid pollen on a low-temperature background significantly increased in those populations the frequency of plants with a higher height and a longer "seed emergence - flowering" period. Taking into account that it was the cold-tolerant parent (the Leader variety) of this hybrid combination that was taller and late-flowering, the revealed change in the structure of the F_2 population indicated that the frequency of plants similar to the cold-tolerant Leader variety was much higher than in the control.

The effectiveness of selection of drought-tolerant genotypes at the stage of mature pollen grains was demonstrated in the following experiment with oil flax. The pollen of K7487 × K7734 hybrid was exposed to a temperature of 35 °C for 1-2 hours

and then used for backcrossing to one of the parents of the hybrid. The control was pollinated with freshly collected pollen (Table 4)

Table 4

Influence of temperature treatment of flax pollen in F_1 K7487 × K7734 on the seed germination in BC_1 K7487 × (K7487 × K7734) on the osmotic medium
(1998-2000)

Pollen treatment	Seed germination, %	Root length, mm
Control	5.0±2.18	1.8±0.40
60 min	19.7±3.39***	1.7±0.17
120 min	34.7±2.57***	2.8±0.28**

, * – differences from the control are significant for $p \leq 0.01$ and 0.001, respectively.

As can be seen from the data in Table 4, heating pollen of hybrid plants influenced both the germination percentage of resulted seeds and the length of the root developed on an osmotic medium. In that case the maximum differences from the control were observed when the pollen was heated for 120 minutes. The obtained results allow drawing a conclusion that selection for heat resistance in a heterogeneous pollen population allows to increase significantly the percentage of drought-tolerant genotypes in the resulted populations of sporophytes [7].

The effectiveness of the technique of mature pollen heating for selection of drought-tolerant genotypes has been shown in other crops, in particular, in castor-bean and sunflower. As for sunflower, it was proved that pollen storage at elevated temperature makes it possible to enhance the heat resistance of the sporophyte generation due to survival of more heat-tolerant gametes (Table 5).

Table 5

Influence of heating of F_1 hybrid pollen on heat resistance in F_2 sporophyte populations of sunflower
(2012-2013)

Crossing combination	Pollen treatment	High temperature treated F_2 seeds sown, pcs.	Germi-nated seeds, pcs.	Seed germination, %
«*virescent*» × «*xantha*»	Control	414	15	3,6±0,92
	60°C/1 hr	144	42	29,2±3,79***
«dichotomous venation» × «burnt leaf»	Control	884	31	3,5±0,62
	60°C/1 hr	912	43	4,7±0,70
	60°C/3 hrs	605	113	18,7±1,59**

*** – differences from the control are significant at $p \leq 0{,}001$.

In some cases, as in the crossing combination "*virescent*" × "*xantha*", the heating of pollen at 60 °C for 1 hour was efficient, whereas for a hybrid with another

parent composition the duration of pollen heating should be increased up to 3 hours to achieve the desired effect [8].

Pollen selection for heat resistance also affected the drought tolerance of F_2 populations. This is evidenced by the comparison of germination of F_2 seeds obtained after pollination with heated pollen, and F_2 seeds obtained after pollination with freshly collected pollen, studying their germination in 15% solution of sucrose and 20% solution of PEG 6000 (Table 6).

Table 6

Influence of heating of F_1 hybrid pollen on seed germination in F_2 populations under osmotic stress conditions
(2013-2014)

Pollen treatment	F_2 seeds, pcs.		Seed germination, %
	total	germinated	
F_2 «dichotomous venation» × «*xantha*», sucrose solution			
Freshly collected pollen	480	43	9.0±1.31
60°C, 1 hr	806	454	56.3±1.75***
F_2 «*virescent*» × «dichotomous venation», PEG 6000 solution			
Freshly collected pollen	156	30	19.2±3.15
60°C, 1 hr	165	97	58.8±3.83***

*** – differences from the control are significant at $p \leq 0.001$

In addition to abiotic factors, male gametophyte can selectively response to biotic factors, in particular, to toxins, which are excreted by pathogens of various plant diseases. In sunflower it was demonstrated that applying pathogen culture filtrate at stylar tissue before pollination has resulted in resistance increase of progeny to Alternaria leaf and stem blight. Moreover, successive pollen selection further improved disease resistance of resulted plants, although to a lesser extent [9].

Our research on pollen selection for resistance to toxins of *Fusarium oxysporum* fungus was carried out on castor-bean plants. In this case, the selective background for germinating pollen was provided by applying a certain concentration of culture filtrate to the stigmas of hybrid plants. The initial heterogeneity of the male gametophytic population for resistance to the fungus toxins was provided by the involvement into crossing those parent components of hybrids which contrasted in the resistance to Fusarium wilt (Table 7).

The results of the studies have demonstrated that this methodical technique has changed the structure of F_2 segregating populations [10]. Thus, at the beginning of flowering, the difference in the number of diseased plants between control and experiment was 13.6% and 27.3% in the F_2 populations of the crossing combinations Khortytskaya 1 × Gibrid ranniy and Khortitskaya 1 × Nebraska, respectively.

Male gametophytic generation can be used to perform selection not only for genotypes tolerant to biotic or abiotic stresses. It is known that significant genetic variability, which is a prerequisite for any type of selection, exists also for the growth rate of pollen tubes under optimal conditions. If a stable relationship exists between pollen tube growth rate and certain traits of the sporophyte, selection at the gamete level will lead to a change in the structure of the sporophytic population.

Table 7

Effect of selection in F_1 of resistant to *Fusarium oxysporum* toxins pollen on resistance of F_2 castor-bean plants to Fusarium disease
(2004-2006)

Treatment	Number of diseased plants, %	
	the start of flowering	the end of the growing season
Khortitskaya 1 × Gibrid ranniy		
Control	21.2±3.93	26.8±4.06
Experiment	7.6±2.39**	16.2±3.32*
Khortitskaya 1 × Nebraska		
Control	54.3±6.59	73.6±5.83
Experiment	27.0±6.48**	53.2±6.91*

*, ** – differences from the control are significant at $p \leq 0.05$ and 0.01, respectively.

It was known from the literature data that fast-growing pollen tubes contribute to the formation of offspring with an earlier development [12]. We conducted experiments on the selection of early flowering genotypes in flax and castor-bean during pollen germination and pollen tube growth. In flax, to ensure only the fastest growing pollen tubes to participate in the fertilization, a half of pistil was cut 40-120 minutes after pollination. In castor-bean, the highest competition between pollen tubes was observed when the pollen grains were loaded to that part of a stigma which was located at a maximum distance from the ovary. The results testified that in a case when the most competitive gametes were involved in fertilization, the frequency of early flowering genotypes in the sporophytic population have increased substantially [13].

However, the rapid growth of pollen tubes and their high competitiveness can be negatively correlated with early flowering, as for example, we observed in spring rapeseed. In this case, in order to realize in the offspring a greater number of early flowering plants, it is necessary to exclude competition between the pollen grains during pollination-fertilization process. This is achieved by applying a small amount of pollen grains to the stigma. As a result of this "limited" pollination, competition between microgametophytes is excluded and practically all genotypes of gametes are manifested in the offspring. Given the negative relationship between the pollen tube growth rate and early flowering, a significantly larger number of early flowering plants will be found in the resulting population than in the case of standard pollination, when an excessive amount of pollen grains are applied to the stigma. Thus, the use of limited pollination in two hybrids of spring rape has ensured in their offspring twice as many plants that bloomed the earliest date [14].

Conclusions

Temperature regimes have been established which allow maintaining a high fertilizing capacity of pollen in oilseeds.

Selection in male gametophytic generation for cold, heat and drought tolerance proved to be successful in oilseed crops, such as rapeseed, oil flax, castor-bean, and sunflower.

It has been demonstrated the possibility of selecting at the pollen level genotypes that are resistant to biotic environmental factors, in particular to Fusarium wilt.

The developed techniques of handling pollen are the basis for the methodological foundation of pollen selection in oilseeds.

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МЕТОДИ ПИЛКОВОГО ДОБОРУ В СЕЛЕКЦІЇ ОЛІЙНИХ КУЛЬТУР

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Представлені результати досліджень з розробки методів мікрогаметофітного добору у олійних культур. Показана можливість пилку олійних культур зберігати свою запліднюючу здатність при різних температурних умовах зберігання. Виявлено, що прогрівання гетерогенної популяції пилку збільшує посухостійкість спорофітних популяцій F_2 у льону олійного та жаростійкість і посухостійкість нащадків, що утворюються, у соняшника. Встановлена можливість добору стійких до холоду генотипів під час проростання пилку і росту пилкових трубок у ріпака ярого. Показана ефективність технології з добору на мікрогаметофітному рівні стійких до фузаріозу генотипів рицини. Встановлено, що у випадку, коли у заплідненні беруть участь найбільш конкурентоздатні гамети, частота генотипів, що квітнуть раніше, у спорофітних популяціях льону та рицини суттєво збільшується. Розроблені методи дозволяють прискорювати процес створення цінного селекційного матеріалу олійних культур.

Ключові слова: мікрогаметофітний добір, пилок, олійна культура, абіотичний фактор, біотичний фактор.

МЕТОДЫ ПЫЛЬЦЕВОГО ОТБОРА В СЕЛЕКЦИИ МАСЛИЧНЫХ КУЛЬТУР

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Представлены результаты исследований по разработке методов микрогаметофитного отбора у масличных культур. Показана возможность пыльцы сохранять свою оплодотворяющую способность при разных температурных условиях хранения. Выявлено, что прогревание гетерогенной популяции пыльцы увеличивает засухоустойчивость спорофитных популяций F_2 у льна масличного, и жаро- и засухоустойчивость образующегося потомства у подсолнечника. Установлена возможность отбора устойчивых к холоду генотипов во время прорастания пыльцы и роста пыльцевых трубок у рапса ярового. Показана эффективность технологии по отбору на микрогаметофитном уровне устойчивых к фузариозу генотипов клещевины. Установлено, что в случае, когда в оплодотворении принимают участие наиболее конкурентоспособные гаметы, частота рано зацветающих генотипов в спорофитных популяциях льна и клещевины существенно увеличивается. Разработанные методы позволяют ускорять процесс создания ценного селекционного материала масличных культур.

Ключевые слова: микрогаметофитный отбор, пыльца, масличная культура, абиотический фактор, биотический фактор.