THE PROBLEM OF SECONDARY CHASMOGAMY IN DISTANT HYBRIDISATION OF WHEAT (*TRITICUMl,.)*

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Abstract: Distant hybridization had been used in plant breeding for a long time to improve varieties of cereal crops. Wild relatives of cultivated wheat possess good adaptation characteristic to different unfavorable environmental factors, both abiotic and biotic. It is well known that Triticum timopheevii Zhuk, has a complex fungal disease immunity. Therefore, this species of wheat is used in breeding programs very often. However, difficulties may arise, such as sterility of FI hybrids, secondary open flowering (secondary chasmogamv), etc.. Secondary chasmogamv is the cause of the uncontrolled cross-pollination between selected samples of wheat.

Reasons for chasmogamous flowering of the following distant wheat hybrid generations, obtained through crossing of Triticum aestivum L. with T. timopheevii have been studied. It has been discovered that secondary chasmogamv phenomenon depends upon neither pollen fertility nor anther size. There is a balance in chromosome number in the genome of the hybrids which are similar to the soft wheat (2n=42). Flowering type does not affect grain setting and the average caryopsis weight without spike isolation. The spike isolation during the flowering stage results in decreasing grain setting for any flowering type and does not affect the grain size. The influence of the female parent variety of soft wheat on the degree of the secondary open flowering in distant hybrids has been revealed.

Key words: wheat distant hybrids, the secondary open flowering, pollen fertility, grain setting in the spike.

Acrop's flowering type determines the characteristics of breeding and seed-production efforts suitable for the crop. Distant hybridization in wheat breeding has been used for a long time. Wild relatives of cultivated wheat have features of good adaptation to various unfavorable abiotic and biotic environmental factors [2, 10]. In this respect, one of the most valuable species is Timofeev's wheat. N.I. Vavilov wrote about it: "... the species of *Triticum timopheevii* is solely interested for its immunity to many diseases” [9]. However, having genome G, that is non-homologous to other species, this plant is very difficult to use in wheat breeding programs. Even after overcoming non-combining ability and sterility of the first generation hybrids, further work is complicated by some unwanted recognition in the offspring, in particular by secondarily chasmogamous flowering [11, 12].

Crop's type of flowering determines the characteristics of breeding and seed production efforts suitable for the crop. Soft wheat is self-pollinated with a small facultative xenogamy (up to 2%) [1, 4, 13]. It allows to carry out breeding and seed-production work with it without isolation. While it retains specific ancestral traits that were typical for ancestral anemophilous forms and lead to the short-term disclosure of lemmas at the time of flowering (primary chasmogamy) and throwing out anthers to expose leftover pollen on long filaments. About 80% of wheat flowers in blooming period open under favorable weather conditions due to lodicule swelling (moderate temperature and excessive humidity).

The time during which the flowers stay open does not last long (around 12 minutes). This process can lead to slight spontaneous cross-pollination [4, 8, 13].

However, in the selection samples obtained with distant hybrids *T. ciestivum* x x *T. timopheevii,* even in later generations (F_7-F_{in}) secondarily chasmogamous flowering is manifested. It is expressed as a prolonged exposure of lemmas in most flowers in the ear. It significantly increases the risk of biological contamination of a variety. Therefore, identifying causes of secondary chasmogamy in distant hybrids *T. ciestivum / T. timopheevii* is a priority task. Its solution will allow using it in breeding programs aimed at developing varieties of wheat with resistance to fungal diseases.

Our research is devoted to studying possible causes of secondary chasmogamy in later generations (F -F) of distant hybrids *T. ciestivum / T. timopheevii*, obtained in 1994 in Altai Research Institute by V.F. Kozlovskaya and M.M. Starostenkova [5].

It is supposed that the secondary chasmogamy can be caused by the following factors: 1 — an imbalance of chromosome count, leading to pollen sterility, 2 — poor anther development, 3 — cytoplasmic male sterility.

Materials and methods

Hybrid combination L-6 [(Zhnitsa x *T. timopheevii) /* Zhnitsa] and L-25 [(Novosibirskaya 67 x I *timopheevii)* x Novosibirskaya 67] were the most appropriate in the conditions of Moscow region. However, in the lines selected from these combinations, secondarily chasmogamous flowering occurs and therefore can be transmitted to selective variants obtained with their participation. To explain reasons of this phenomenon the authors selected lines contrasting in pollen fertility that turn out to be populations because of secondary chasmogamy (three lines with increased and three lines with reduced fertility from each combination) and their parental forms. L-6-7, L-6-10, L-6-12 are lines with increased pollen fertility for combination L-6 [(Zhnitsa x *T. timopheevii)* x Zhnitsa]. L-6-6, L-6-15 and L-6-19 are lines with low pollen fertility. L-25-22, L-25-23 and L-25-26 are lines with increased pollen fertility for combination L-25 [(Novosibirskaya 67 x x *T. timopheevii)* x Novosibirskaya 67]. L-25-21, L-25-24, L-25-27 are lines with low pollen fertility. Secondarily chasmogamous flowering appears to different degrees in each line. Therefore, normally flowering plants and plants with secondary chasmogamy were selected in each line. The experiment comprised populational lines used for calculating the percentage of chasmogamous plants at a plot, as well as the selecting variants differing in their flowering type.

Investigations were carried out at the Department of plant breeding and seed production of field crops and at the Centre for Molecular Biotechnology of Russian State Agrarian University - Moscow Agricultural Academy named in honour of K.A. Timiryazev in 2010-2011. Test samples were planted at the Plant breeding station named in honour of P.I. Lisitsyn. In 2010, the total area of plots of initial populational lines was 1.5 m^2 while in 2011 - 1 m^2. Offspring of selected plants with different types of flowering within each line were hand-seeded. Cultural practices common for Non-chernozem zone were applied.

Weather conditions prevailing during the growing season were quite different in the two years of research. In 2010, at the beginning of flowering one could observe the most severe drought accompanied by extremely high temperatures followed by abundant rainfall. It led to the formation of grains in ear very low in weight. In 2011, on the contrary, at the beginning of growing and flowering seasons one could observe a bad drought coupled with high temperatures. The end of flowering coincided with heavy rainfall. Maturation proceeded in the conditions of marked moisture deficit with the average daily temperatures being high.

Chromosome counting was performed at metaphase plates [7]. Pollen fertility was identified by the acetocarmine method [6]. The dimensions of anthers were measured using a stereoscopic microscope MBS-3 in plants with secondary chasmogamy and in normally flowering plants within the limits of each line. During flowering, spikes of plants with two types of flowering were isolated in order to study the effects of the isolation on set of seed and caryopsis mass. The set of seed was defined as the ratio of the number of grains to the number of developed flowers. Data were modified and adjusted and then processed with the analysis of variance using the program «DIANA» [3].

Results and discussion

In 2010, high occurrence level of secondarily chasmogamous flowering in the parental forms of Zhnitsa and lines of L-6 combination with its participation (about 40%) was recorded. In the secondary combination of L-25, only two lines (L-25-22 and L-25-24) had a high percentage of secondary chasmogamy (10% and 20% respectively). In 2011, the occurrence of secondary chasmogamy was lower in all lines. In L-6 combination it ranged from 4% to 22%, in Zhnitsa - 1.2%, in L-25 combinations from 0 to 3%. Moreover, in the parental form of Novosibirskaya 67, the secondary chasmogamy was not discovered. Thus, the influence of maternal variety of soft wheat is obvious.

Chromosome number count in all studied lines of distant hybrids showed that all of them had a diploid number of chromosomes that is typical for *T. ciestivum* — 42. Aneuploidy was not recorded.

Pollen fertility study showed that all lines had absolutely normal pollen, though they differed in the fertility value. The lowest fertility value is recorded in the L-6-19 line 79%. This is enough to fertilize one ovule in a flower. In this case, none of the years of studies revealed a correlation between pollen fertility and percentage of secondarily chasmogamous plants at a plot.

Anther size in studied lines of distant hybrids varied considerably and was either within the range of the parental forms, or much larger. There was no correlation between the size of anthers, on one hand, and the percentage of secondarily chasmogamous plants at the plot and pollen fertility - on the other. The comparison of anther sizes in the secondarily chasmogamous and normally flowering plants within each line showed no significant differences in this trait between plants with different types of flowering.

Set of seed without spike isolation

In all lines of both combinations, the set of seed and the mass of one caryopsis without prior isolation of ears in the flowering stage were determined (Table 1).

In L-6 combination in 2010 there were marked differences between secondarily chasmogamous plants in studied lines and no marked differences in normally flowering plants. Two-factor analysis of variance showed that the set of seed in normally flowering plants was significantly higher than that in the secondarily chasmogamous plants. In 2011, similar results on the set of seed in the studied lines were obtained. But there were no significant differences between plants with different types of flowering within each line.

As for the L-25 combination, it should be noted that in 2010 the lines did not differ from each other in the percentage of the set of seed in the ear in secondarily chasmogamous plants, while in normally flowering plants lines differed significantly. In 2011, the differences between lines were observed in plants with both types of flowering. Two-factor analysis of variance did not reveal significant differences in the set of seed between plants with different types of flowering during two years of study.

Table 1

Set of seed and weight of caryopsis in plants with different types of flowering in studied lines without spikes isolation

Line	Set of seed,%				Weight of one caryopsis, mg			
	Secondarily chasmogamous plants		Normally flowering plants		Secondarily chasmogamous plants		Normally flowering plants	
	2010	2011	2010	2011	2010	2011	2010	2011
L-6 combination [(Zhnitsa x *T. timopheevii)* x *Zhnitsa]*								
L-6-6	67	52	74	65	29	41	36	36
L-6-7	53	69	54	68	21	34	29	36
L-6-10	62	69	70	71	24	40	31	40
L-6-12	56	76	68	66	24	32	35	30
L-6-15	44	56	63	60	28	41	29	37
L-6-19	66	71	64	71	21	36	25	34
HCP_{05}	10.2	7.0	14.1	4.9	11.2	5.2	10.6	6.1
L-25 combination [(Novosibirskaya 67 x *T. timopheevii)* x *Novosibirskaya 67]*								
L-25-21	-	89	-	71	-	38	-	37
L-25-22	61	66	64	61	20	34	32	38
L-25-23	-	76	-	72	-	33	-	36
L-25-24	53	66	65	67	17	35	32	36
L-25-26	68	86	82	72	22	32	31	32
L-25-27	-	-	-	-	-	-	-	-
HCP_{05}	18.9	7.0	6.0	4.9	7.4	5.2	6.6	6.1

Both hybrid combinations showed no differences in one caryopsis weight between the lines in any type of flowering in 2010 and 2011. However, in 2010 it was found that grains of normally flowering plants are significantly larger than those of open-flowering plants. In 2011, no differences were found between the plants with two types of flowering.

Thus, in the studied combinations of distant hybrids there was no significant effect of the flowering type of the set of seed and caryopsis weight without preliminary isolation of spikes.

Set of seed with spike isolation

Spike isolation in the flowering stage can lead to the lack of grains in case of sterile pollen or cytoplasmic male sterility.

In our experiments, a complete lack of grains in isolated spikes has never been recorded. It indicates the absence of the above mentioned causes in the studied lines of distant hybrids.

Nevertheless, we have evidence to state a significant influence of the isolation on the set of seed in distant hybrids of wheat (Table 2).

Table 2

Set of seed and caryopsis weight in plants with different types of flowering in studied lines with spike isolation, 2011

Line	Set of seed, %				One caryopsis weight, mg			
	secondarily chasmogamous plants		normally flowering plants		secondarily chasmogamous plants		normally flowering plants	
	isolated	intact	isolated	intact	isolated	intact	isolated	intact
L-6 combination [(Zhnitsa x *T. timopheevii)* x *Zhnitsa]*								
L-6-6	44	52	54	65	42	41	39	36
L-6-7	52	69	56	68	34	34	36	36
L-6-10	48	69	57	71	40	40	41	40
L-6-12	66	76	62	66	30	32	28	30
L-6-15	39	56	42	60	41	41	39	37
L-6-19	54	71	62	71	35	36	34	34
*L-25 combination [(Novosibirskaya 67 * T. timopheevii)* x *Novosibirskaya 67]*								
L-25-21	72	89	60	71	38	38	37	37
L-25-22	59	66	59	61	32	34	33	38
L-25-23	71	76	58	72	35	33	34	36
L-25-24	52	66	55	67	33	35	36	36
L-25-26	82	86	67	72	30	32	30	32
L-25-27	-	-	57	67	-	-	37	34
Zhnitsa	62	74	62	74	34	33	34	33
Novosibirskaya 67	61	70	61	70	32	33	32	33
T. timopheevii	59	80	59	80	25	26	25	26
HCP_{05}	8.0	6.7	7.1	5.0	6.4	5.2	6.9	5.7

Spike isolation of previously selected secondarily chasmogamous and normally flowering plants within each line showed that both with isolation and without it the set of seed varied greatly in different lines. Thus, in lines L-6-6-6 and L-6-15, significantly low set of seed in comparison with the maternal variety Zhnitsa was recorded regardless of flowering type and isolation. In another combination, similar data were obtained for lines L-25-21 and L-25-26. On the contrary, they were found to have higher set of seed than the maternal variety Novosibirskaya 67, regardless of the isolation. However, the two-factor analysis of variance showed that in case of spike isolation the set of seed reduces significantly in comparison with the intact plants in plants with both types of flowering.

Spike isolation at the blooming stage does not affect the formation of the caryopsis weight in plants with both types of flowering during two years of research.

Conclusions

Studied later generations of distant hybrids of *T. ctestivum / T. timopheevii* have a genome that is balanced in the chromosome number. It is similar to that of soft wheat (2n = 42).

Distant hybrids have normally developed anthers, they are often larger than those of their parents.

The studied lines of distant hybrids *T. ctestivum / T. timopheevii* have high pollen fertility. They are found to have no cytoplasmic male sterility.

Isolation reduces the set of seed in case of any type of flowering (secondary chasmogamy and normal flowering).

Spike isolation during flowering does not influence the size of a caryopsis.

The influence of the soft wheat's maternal variety on the secondary chasmogamy in hybrids is revealed.

Thus, the question of the causes of secondary chasmogamy in later generations of distant hybrids *T. ctestivum* / *T. timopheevii* remains open.

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ПРОБЛЕМА ВТОРИЧНОЙ ХАЗМОГАМИИ В ОТДАЛЕННОЙ ГИБРИДИЗАЦИИ ПШЕНИЦЫ *(TRITICUM*L.)

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Аннотация: отдаленная гибридизация используется в селекции довольно давно для улучшения сортов полевых культур. Дикие сородичи культурной пшеницы обладают признаками хорошей адаптации к различным абиотическим и биотическим факторам среды. Пшеница Тимофеева (Triticum timopheevii Zhuk.) обладает комплексным иммунитетом к грибным болезням, поэтому часто используется в селекционных программах. Однако возникают трудности, такие как стерильность гибридов F1, вторичное открытое цветение (вторичная хазмогамия) и др. Вторичная хазмогамия приводит к неконтролируемому перекрестному опылению и, как итог, к популятивности сортообразцов.

В данной работе изучены предполагаемые причины вторичного хазмогамного цветения поздних поколений отдаленных гибридов пшеницы, полученных от скрещивания Triticum aestivum L. с T. timopheevii Zhuk. Показано, что вторичная хазмогамия у них не зависит от фертильности пыльцы и размеров пыльников. Изучаемые поздние поколения отдаленных гибридов имеют сбалансированный по числу хромосом геном, сходный с мягкой пшеницей (2n=42). Тип цветения не влияет на завязываемость и среднюю массу зерновки без изоляции колосьев. Изоляция колосьев в фазу цветения приводит к снижению завязываемости зерен при любом типе цветения и не влияет на формирование крупности зерен. Выявлено влияние материнского сорта мягкой пшеницы на степень вторичного открытого цветения отдаленных гибридов.

Ключевые слова: отдаленные гибриды пшеницы, вторичное хазмогамное цветение, фертильность пыльцы, завязываемость зерен в колосе.

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