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Study of Fertility and Cytogenetic Variability in Androgenic Plants (R_0 and R_1) of the Alloplasmic Introgression Lines of Common Wheat

T. S. Osadchaya[a], N. V. Trubacheeva[a], L. A. Kravtsova[a], I. A. Belan[b], L. P. Rosseeva[b], and L. A. Pershina[a, c, *]

[a] *Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*
[b] *Siberian Agricultural Research Institute, Omsk, Russia*
[c] *Novosibirsk State University, Novosibirsk, Russia*
**e-mail: pershina@bionet.nsc.ru*

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Abstract—Anther culture is one of the methods for obtaining doubled haploid (DH) lines of wheat, widely used in genetics and breeding. The cytogenetic instability in R_0 plants, leading to a decrease in fertility or sterility, can be a limitation of this method. In this study, we have investigated the fertility of R_0 and the fertility and cytogenetic variability of R_1 in the alloplasmic introgression lines of common wheat in order to develop cytogenetically stable DH lines with introgressions from different species. Lines 311/134, 311/FL, and 311/IR with the cytoplasm from *H. vulgare* were studied. The 311/134 line carries the wheat–rye 1RS.1BL and the wheat–wheatgrass 7DL-7Ai translocations; the 311/FL line has the 1RS.1BL translocation and probably introgressions from *A. glaucum*; and the 311/IR line has the wheat–rye 1RS.1BL and wheat–*Ae. speltoides* T2B/2S#2 translocations. Green seedlings developed in the anther culture of all the lines. The differences between the lines in their ability for androgenesis and in the level of fertility in R_0 and R_1 have been revealed. Depressed androgenesis, low fertility, and high aneuploidy were observed in 311/IR. It has been proposed that the reason for this is the cytogenetic instability of the gametes, caused by the *Gc* genes located on T2B/2S#2. Among the 311/134 and 311/FL R_1 plants, grown from low seed-set R_0 plants, 63.3% were aneuploids. Fertile R_0 regenerant plants that segregated in R_1 by fertility and chromosome numbers were identified. It was demonstrated that the DH lines are best developed from high-fertility R_1 plants with $2n = 42$ and a high fertility level, irrespective of the fertility level of R_0.

Keywords: alloplasmic introgression lines (*H. vulgare*)–*T. aestivum*, anther culture, androgenesis, dihaploid lines, fertility, cytogenetic instability

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INTRODUCTION

The development of DH lines, based on haploid plants with the doubled number of chromosomes, is an important and widely used technology for genetics and breeding (Forster and Thomas, 2005). Since such lines are homozygous, they can be quickly analyzed; thus, the time of selecting genotypes with the desired characteristics can be reduced and the breeding process can be accelerated (Germana, 2011). Such lines are a unique material for the detailed study of the many traits in plants, including quantitative traits (Hussain et al., 2012).

Interspecies hybridization, which resulted in the elimination of the chromosomes of the species, crossed with the wheat at the early stages of embryogenesis and the development of haploid wheat seedlings, were used for the formation of the DH lines of wheat (Niroula and Bimb, 2009). Another approach is based on the in vitro induction of androgenesis: the development of embryo-like structures from microspores and then androgenic seedlings in anther cultures (Barnabas et al., 2001), or isolated microspores (Liu et al., 2002). The efficiency of these methods is estimated by the number of unique lines, required for further genetic or breeding studies (Oleszczuk et al., 2011). Limitations in the formation of DH lines by microspores and anther culturing related to the manifestation of the gametoclonal variability characteristic for plants regenerated in vitro from gametic cells. The genetic mechanisms of gametoclonal variation include changes in the chromosome number (polyploidy, aneuploidy) and their structure (duplications, translocations, inversions, deletions), and molecular changes in the nuclear, mitochondrial, and chloroplast genomes (Veilleux, 1998).

A typical manifestation of the gametoclonal variability associated with deletions in the chloroplast genome is the development of chlorophyll-defective

seedlings (albino plants) (Jane and Lorz, 1995). Mutations in the nuclear genome and cytogenetic variability lead to the sterility or reduced fertility of dihaploid plants (Hu and Huang, 1987). However, the spontaneous doubling of the chromosome number in regenerated seedlings restores their fertility, enabling the treatment of androgenic plants with colchicine during the production of DH lines (Osadchaya et al., 2015). Cytogenetic variability in dihaploids is especially apparent when genotypes of hybrid origin, characterized by cytogenetic instability in meiosis, are used as the donor plants for the cultivation of anthers and microspores (Oleszczuk et al., 2011). However, the genotypes of hybrid origin, carrying alien genetic material, are important objects for the formation of DH lines, since the combination of a series of target genes, transferred from different parental genotypes, including those responsible for the resistance to stress factors, can be fixed (Joshi and Nayak, 2010).

In our previous study (Osadchaya et al., 2015), the alloplasmic introgression DH lines (*H. vulgare*)-*T. aestivum* with wheat-alien translocations were obtained as a result of anther cultivation. The study of these lines showed the prospects of their use in the breeding process and genetic studies. However, a number of issues related to the need for a more effective selection of androgenic plants for the formation of highly fertile 42-chromosome DH lines remained. Thus, in the previously studied (*H. vulgare*)–*T. aestivum* introgression lines, clusters (families) developed from one embryo-like structure instead of single R_0 seedlings (Osadchaya et al., 2015). This complicated the cytogenetic analysis of individual R_0 plants in the course of the selection for the formation of DH lines.

The aim of this work was to investigate manifestations of fertility in androgenic R_0 plants, regenerated in the anther culture of the alloplasmic introgression lines of common wheat (*H. vulgare*)–*T. aestivum*, and the level of fertility and cytogenetic variability of R_1 plants. This is necessary for the identification of highly fertile 42-chromosome plants, which are sources for the formation of dihaploid lines, combining the genetic material of different species.

MATERIALS AND METHODS

Plant Material

Three promising lines derived from hybrid populations produced at the Siberian Agricultural Research Institute (Omsk) were used in the study. The alloplasmic introgression line of common wheat Lutescens 311/00-22-4, which carries the cytoplasm of cultivated barley *Hordeum vulgare* L. and the wheat–rye 1RS.1BL translocation, was taken as the maternal genotype in obtaining hybrids. The origin of this line was reported earlier (Pershina et al., 2013). The following lines were used as the paternal genotypes: the Lutescens 134/03-10 line, the breeding record of which contained common wheat Omskaya 37, a carrier of the wheat–rye 1RS.1BL and wheat–wheatgrass 7DL-7Ai translocations (Belan et al., 2012a; Belan et al., 2015); the line of the Filatovka variety obtained using *Agropyrum glaucum* (Stepochkin et al., 2012); and the line of the Yrym variety, characterized by the high field resistance to leaf pathogens throughout ontogeny (Belan et al., 2012b). According to the preliminary data of E.I. Gul'tyaeva, the Yrym variety has the *Lr35/Sr39* genes from *Ae. speltoides*, indicating the presence of the T2B/2S#2 translocation (Friebe et al., 1996). The lines for anther cultures during the formation of androgenic green plants were formed from the plants with the best productivity, which were resistant to leaf pathogens under field conditions. According to the methods described below, the lines were tested for the presence of the wheat–rye 1RS.1BL and wheat–wheatgrass 7DL-7Ai translocations (Table 1).

Table 1. The origin of parental lines

Lines	Origin of genotypes
311/134	Lutescens 311/00-22-4/Lutescens 134/03-10
311/FL	Lutescens 311/00-22-4/Filatovka
311/IR	Lutescens 311/00-22-4/Yrym

Anther Culture

The donor plants for the anther cultures were grown in a hydroponic greenhouse. The conditions of anther pretreatment, their isolation and culture conditions were optimized earlier (Pershina et al., 2013). Anthers were cultured in a PII induction medium (Chuang et al., 1978), supplemented with 0.75 mg/L of 2,4-D sucrose (45 g/L) and maltose (45 g/L), and Bacto Difco agar (8 g/L). In order to regenerate seedlings from embryo-like structures (ESs) a Gamborg medium (Gamborg and Eveleigh, 1968) without growth regulators was used. The anthers were cultured at $t = 29°C$ without light and ESs were cultured at $t = 24°C$ in continuous light. The features of androgenesis were evaluated by the frequency of the anther, formed ESs, and frequency of the ESs and green seedlings to 100 cultured anthers. The green seedlings were transferred in soil at the stage of three leaves and grown as previously described (Osadchaya et al., 2015). The treatment of androgenic plants with colchicine was not performed. Fertility studies were done on plants with spontaneously doubled chromosomes.

Study of Androgenic R_0 and R_1 Plants, and the Formation of Dihaploid Lines

The fertility rates in R_0 regenerates and their self-fertilized offspring R_1 were evaluated by the number of

Table 2. Cultivation of anthers from the introgression genotypes of common wheat

Parental lines	The number of cultivated anthers	Productive anthers		Embryo-like structures		Green seedlings	
		total	frequency, %[#]	number	frequency, %[#]	total	frequency, %[◇]
311/134	509	91	17.9	285	56.0	149	29.2
311/FL	359	48	13.4	222	61.8	95	26.5
311/IR	766	51	6.6***	98	12.8***	113	14.7*

[#] per 100 cultivated anthers; [◇] per total number of seedlings. Differences from lines 311/134 and 311/FL are significant at $*p < 0.05$ and $*** p < 0.001$.

grains per ear, according to the method of P. Sinha et al. (2013) with our modifications. Fully sterile (FS)—no grains; partially sterile (PS)—from 1 to 5 grains; partially fertile at a low level (PF–)—from 6 to 10 grains; partially fertile (PF+)—from 11 to 19 grains; fertile (F)—20 to 29 grains; and fully fertile (FF)—more than 30 grains per ear. R_1 plants of all lines were grown in a greenhouse in the same growing season. The data were processed using the Statistica v.7.0.61.0 program.

The analysis of the chromosome number was carried out according to the standard methods of sample preparation according to Feulgen. SCAR-marker iag95, linked to the *Lr26* and *Sr31* genes, localized on the short arm of the rye chromosome 1R was used for confirmation of the presence of the 1RS.1BL translocation in the initial lines and R_0 and R_1 regenerates obtained, based on these lines (Mago et al., 2002). The SCAR-marker scm265, linked with the *Lr19* gene localized on the *Ag. elongatum* (Host.) Beauv chromosome 7AgL was used for the identification of the wheat–wheatgrass translocation 7DL-7Ai (Gupta et al., 2006).

For the selected studies, the DH lines were formed from the 42-chromosome R_1 plants, exhibiting full fertility. The DH lines for the genetic studies and further selection for breeding were formed from the R_0 and R_1 of all fertile plants.

RESULTS

Formation of R_0 Plants and Peculiarities of Their Fertility

Anther cultivation revealed the ability for androgenesis of lines 311/134, 311/FL, and 311/IR, and as a result green seedlings (R_0) were obtained. At the same time, for line 311/IR such androgenesis indices as the frequency of the productive anthers, the formation rate of embryonic structures, and the regeneration frequency of the green seedlings, estimated per 100 cultured anthers, were significantly lower than the same indices for the other two lines: 311/134 and 311/FL (Table 2).

As in the previous studies (Osadchaya et al., 2015), mainly clusters rather than individual seedlings developed from the ESs. These clusters were separated into individual seedlings and planted in the soil. At the end of the growing season and harvesting of the R_0 plants, their exact number in the cluster could not always be determined; therefore, each ear was attributed to a single plant.

The results of the study of the R_0 regenerants of each line based on the level of fertility, estimated according to the number of grains per ear and distributed in six groups: FS, PS, PF^-, PF^+, F, and FF are shown in Table 2.

As follows from the data in Table 2, the lines differed by the level of fertility of the R_0 regenerants. Thus, line 311/IR developed mainly completely sterile plants (85.6%); only one of them contributed to the fertile group and fully fertile plants were not discovered. In line 311/134 full sterile was characteristic for half of the studied 149 plants (51.7%). The lowest frequency of sterile plants (25%) was detected for the line 311/FL. Plants FS, PF^-, PF^+, F, and FF forming a different number of grains per ear and, respectively, related to certain groups by the fertility level were developed by lines 311 and 311/134/FL with approximately the same frequency (Table 3).

The majority of sterile plants were narrow-leaved, with short stems and ears. Selective cytological analysis showed that they are haploid ($n = 21$). In addition, tall plants with normally developed ears were identified among the sterile plants.

The Fertility Rate and Chromosome Number of R_1 Plants—Progenies of Low-Fertility R_0 Regenerants

The expression of fertility was studied and the the chromosome number in the R_1 plants grown from the seeds of the low fertile R_0 regenerants (lines 311/134 and 311/FL), which formed not more than 10 grains per ear (PS and PF- groups) was analyzed. The data shown in Table 4 demonstrate, that among the R_1 plants grown from the seeds of partially sterile and partly fertile R_0 regenerants, completely sterile plants and plants with different levels of fertility, including fertile and fully fertile plants, developed. The variability in the chromosome number was characteristic for

Table 3. Fertility of R_0 regenerants

Parental lines	The number of studied plants	The number and frequency, % of R_0 plants with different fertility levels					
		FS	PS	PF–	PF+	F	FF
311/134	149	77 (51.7***)	11 (7.4)	5 (3.3)	16 (10.7)	21 (4.1)	19 (12.8)
311/FL	92	23 (25.0)	14 (15.2)	2 (2.2)	20 (21.7)	17 (18.5)	16 (17.4)
311/IR	111	95 (85.6***)	2 (1.8)	2 (1.8)	11 (9.9)	1 (0.9)	0 (–)

The difference in comparison with the frequency of the plants of the same line that set seed is significant at *** $p < 0.001$. Hereinafter, FS—fully sterile; PS—partially sterile; PF—partially fertile at a low level; PF+—partially fertile; F—fertile; FF—fully fertile.

Table 4. Fertility levels and the chromosome number in R_1 plants derived from R_0 plants of the low-fertility lines 311/FL and 311/134

Characteristics	Fertility of R_0 plants	The number of plants examined	Fertility in R_1 plants					
			FS	PS	PF–	PF+	F	FF
The number and frequency, % of plants	FS and PF– lines 311/FL and 311/134	49	10 (20.4)	7 (14.3)	4 (8.1)	8 (16.3)	4 (8.2)	16 (32.6)
2*n*			37, 39, 40, 41, 42	40, 41, 40 + t, 41 + t	39, 40, 41, 42	40, 40 + t, 41, 42	40, 42	43, 44, 42*
The number and frequency, % of plants	PF+ lines 311/IR reg49p1 reg49p2 reg226p1	29	1 (3.4)	0 (–)	5 (17.2)	12 (41.4)	9 (31.0)	–
2*n*			42	–	43	41, 43, 42	43, 45, 42*	–

* Plants—sources of dihaploid lines included in the breeding.

each R_1 plant group ranked according to the level of fertility. Cytotypes with chromosome number $2n = 37$, 39, 40, 41, and 42 were detected among the ten fully sterile plants. Among the 11 partly sterile plants, cytotypes with chromosome number $2n = 40$, 40 + t, 41, 41 + t were detected. Each of the four partially fertile plants had a different chromosome number: $2n = 39$, 40, 41, and 42. Among the eight plants with PF+ cytotypes were identified with $2n = 40$, 40 + t, 41 and two plants were identified with $2n = 42$.

The group of fertile plants (20 to 29 grains per ear) included two plants with $2n = 40$ and two euploids ($2n = 42$). Of the 16 fully fertile plants (set of 30 grains and more), 12 plants had $2n = 42$, one plant had $2n = 44$, and 3 plants had $2n = 43$. The dihaploid lines were formed using 42-chromosome plants with fully fertility. Based on the PCR analysis, we established that five of the DH 311/FL lines were carriers of the *Lr26/Sr31* genes (it indicates the presence of the wheat–rye 1RS.1BL translocation), and two dihaploid lines 311/134 were carriers of the *Lr26/Sr31* and *Lr19/Sr25* genes, localized on a segment of wheatgrass chromosome in the 7AgL translocation. The formed lines in two subsequent self-pollinated generations (R_2 and R_3) remained fully fertile. The selective cytogenetic analysis among the R_2 and R_3 plants identified only 42-chromosome plants. In this part of the work, R_1 plants that are progenies of the three partially fertile plants from the clusters of two regenerants reg49 and reg22 of line 311/IR, characterized by a low number of fertile plants (R_0) regenerated in anther cultures were also studied (see Table 4; designation of plants—sources of R_1 lines: reg49p1, reg49p2, reg226p1). These plants carry the wheat–rye 1RS.1BL translocation, as was determined by the PCR analysis, which identified the linked genes located on 1RS.

The progenies of R_0 regenerants with partial fertility of line 311/IR in R_1 generation were segregated based on the manifestation of fertility into partially

Table 5. Fertility levels and the chromosome number of R_1 plants derived from two clusters of R_0 of line 311/134 and two R_0 plants of line 311/FL

Fertility of R_0	The number of R_1 plants and the number of the line	The number and frequency, % of R_1 plants with the determined fertility levels					
		FS	PS	PF–	PF+	F	FF
PF– reg208p1	9 311/134	4 (44.4)	3 (33.3)	(11.1)	0	1 (11.1)	0
PF+ reg208p2	52 311/134	7 (13.4)	10 (19.2)	9 (17.3)	20 (38.5)	4 (7.7)	2* (3.8)
					$2n = 41$ $2n = 42$		$2n = 42$
F reg208p3	62 311/134	3 (4.8)	24 (38.7)	6 (9.6)	14 (22.5)	15 (24.2)	0
						$2n = 42$ $2n = 43$	
PF+ reg317p1	20 311/134	0	0	0	8 (40.0)	8 (40.0)	4 (20.0)
						$2n = 42$	
F reg317p2	20 311/134	0	0	0	4 (20.0)	8** (40.0) $2n = 42$	8* (40.0) $2n = 42$
FF reg317p3	21 311/134	0	0	0	1 (4.8)	6** (28.6)	14* (66.6)
						$2n = 42$	$2n = 42$
F reg61p1	19 311/FL	0	0	0	1 (5.3)	12** (63.2)	6* (31.5)
						$2n = 42$	$2n = 42$
F reg106p1	19 311/FL	2 (10.5)	1 (5.3)	1 (5.3)	3 (15.8)	9** (47.3)	3** (15.8)
						$2n = 41$ $2n = 42$	$2n = 42$

* Plants—sources of the DH lines included in the breeding. ** Plants—sources of the DH lines used in the subsequent study.

fertile (PF– and PF+) and fertile groups (see Table 4). Of the 29 studied plants, one plant with $2n = 42$ was completely sterile. Most of the plant set seeds were aneuploid with the chromosome number 41, 43, and 45. Only two plants from the fertile group and three plants from the partially fertile group were 42-chromosome plants. The two DH lines exhibiting full fertility in R_2 under field conditions and demonstrating complete resistance to leaf pathogens were formed based on these plants.

Fertility Level of R_1 Plants of Lines 311/134 and 311/FL

Three plants derived from two clusters of R_0 regenerants, reg208 and reg317 (plants designated as reg 208p1, reg208p, and reg208p3 and accordingly reg317p1, reg 317p2, and reg317p3) were used for the formation of the R_1 generation of line 311/134. According to the data of the PCR analysis, these plants carry the linked *Lr26/Sr31* genes and the *Lr19* gene, indicating the presence of the 1RS.1BL + 7DL-7Ai translocations.

The plant reg208p1 was characterized by partial low-level fertility, reg208p2 was partially fertile, and reg208p3 was fertile. The plant reg317p1 was partially fertile, reg317p2 was fertile, and reg317p3 was fully fertile. It was found that the lines formed from the reg208 regenerant differed by the manifestation of fertility from the reg317 lines (Table 5). For all three lines of the regenerant reg208, regardless of the fertility level of the parent plants, completely sterile plants and partially fertile plants developed in the R_1 generation (PS and PF–).

Even among the progenies of the fertile reg208p3 plants only 24.2% of the fertile genotypes were detected; the rest of the plants had lower fertility levels (see Table 5). Two 42-chromosome fully fertile plants

were found among the plants of the R_1 line formed from the reg208p2 plant (PF+). The selective cytological analysis revealed the presence of aneuploids with $2n = 41$ and $2n = 43$ among the group of plants that were partially fertile and fertile.

The lines of R_1 generation formed from the reg317 regenerant were characterized by a relatively high fertility level (see Table 4). Thus, among the R_1 plants of the reg317p1 line (the parent plant R_0 was partially fertile PF+) and line reg317p2 (parent plant R_0 was fertile), as well as among the plants of line reg317p3 (the parent plant R_0 was fully fertile), sterile plants and plants with low sets of seeds (PS and PF-) were not detected. The selective cytological analysis of the R_1 plants of these lines revealed only the presence of plants with $2n = 42$. The DH lines, which over three years (the R_2, R_3, and R_4 generations) were trialed breeding tests, exhibiting full fertility and resistance to fungal pathogens, were formed based on 42-chromosome plants with the manifestation of full fertility (the plant groups used for the formation of such lines are shown in Table 5).

Different levels of fertility were observed in the R_1 generation among the self-pollinated offspring of different fertile R_0 plants of line 311/FL. The data for the R_1 lines formed based on the reg61p1 and reg106p1 plants carrying the wheat–rye 1RS.1BL translocation are shown in Table 5. Line per61p1 was represented by only 42-chromosome plants with high fertility levels. Line reg106p1 segregated into plants with different fertility levels: from sterile plants to plants with fully fertile. Among the fertile plants, both euploid and aneuploid cytotypes were identified.

DISCUSSION

Line 311/134, used in this study, combines two translocation, 1RS.1BL and 7DL-7Ai, carrying clusters of linked genes responsible for resistance to fungal pathogens *Lr26/Sr31/Yr9/Pm8* (Singh et al., 1990) and *Lr19/Sr25* (Liu et al., 2010), respectively. Due to the additive effect of the *Lr26* and *Lr19* genes and the functioning of the *Sr25* gene, the common wheat varieties Omskaya 37, Omskaya 38, and Omskaya 41 produced by the authors of a project at Siberian Agricultural Research Institute (Belan et al., 2012a; Belan et al., 2015) have strong resistance to leaf and stem rust, including the stem rust race Ug99 + *Sr24* (TTKST) (Belan et al., 2012b). Line 311/FL with the paternal genotype of the winter wheat variety Filatovka, obtained with the participation of wheatgrass *A. glaucum* (Stepochkin et al., 2012), also carries the 1RS.1BL translocation. The hybrid line 311/IR with the 1RS.1BL translocation also has a wheat–*Ae. speltoides* T2B/2S#2 translocation (with the *Lr35ISr39* genes).

The efficiency of the production of the dihaploid lines using the cultivation of anthers is determined by the successful completion of all stages of androgenesis with the formation of green seedlings (R_0) and the formation of 42-chromosome plants (spontaneously induced under cultivation or as a result of the treatment with colchicine), characterized by a high fertility level. It was found that the genotype of the donor plant has a determining influence on the androgenesis (development of embryoids from microspores, regeneration of all seedlings from embryoids, and the development of green seedlings), even with the optimization of all the conditions for anther cultivation (Konieczny et al., 2003; Tersi et al., 2006). The results of this study showed that line 311/IR has a decreased ability for androgenesis in comparison with the two other studied lines (see Table 2). Thus, the frequency of the anthers, which formed embryo-like structures, and the frequencies of ES development and regenerated green seedlings for this line were significantly lower than for lines 311/134 and 311/FL.

As mentioned above, all the studied lines are carriers of the wheat–rye 1RS.1BL translocation and the *H. vulgare* cytoplasm. Previously, we demonstrated the stimulating effect of the barley cytoplasm and the wheat–rye 1RS.1BL translocation on the manifestation of the traits of androgenesis in the alloplasmic genotypes (*H. vulgare*)-*T. aestivum* (Pershina et al., 2013; Osadchaya et al., 2015), including the manifestation in the presence of the wheat–wheatgrass translocation 7DL-7Ai (Osadchaya et al., 2015). It is important to note this, since in euplasmic (with wheat cytoplasm) genotypes, the 7DL-7Ai translocation (Sibikeeva et al., 2004), also in combination with the 1RS.1BL translocation (Osadchaya et al., 2015), has a strong inhibitory effect on the expression of androgenesis in anther cultures. In line 311/134 (carrier of 1RS.1BL 7DL-7Ai), the values of all the studied parameters of androgenesis remain at the level of these indicators, identified in line 311/FL (1RS.1BL carrier). Thus, in the genotypic environment of line 311/134, the negative impact on 7DL-7Ai on the androgenesis is suppressed.

As for line 311/IR, which carries the wheat–rye 1RS.1BL and wheat–*Ae. speltoides* T2B/2S # 2 (with *Lr35/Sr39* genes) translocations, its lower indices of androgenesis (see Table 2) can probably be explained by the cytogenetic instability of the donor plants of anther for cultivation. In this study, the cytogenetic analysis of the donor plants was not carried out. However, the long-term study of the authors revealed the lack of prospects of using the Yrym variety in crosses with other common wheat genotypes, for the transfer of genes which determine the resistance to leaf pathogens, from this variety into a new genotypic environment. The hybrid lines obtained using Yrym were characterized by low productivity due to the sterility or

reduced fertility of the plants. The selective cytogenetic analysis detected plants with cytogenetic alteration (our unpublished data).

We can assume that in the genetic material of *Ae. speltoides*, which carries the Yrym variety and which was transferred in line 311/IR, there are gametocidal genes. This is confirmed by the fact that the gametocidal *Gc* genes are localized in chromosome 2S of *Ae. speltoides*. In a hemizygous state, these genes induce chromosome breaks, leading to the appearance of chromosome fragments and bridges in microspores not carrying the *Gc* genes during the interphase of mitosis (Nasuda et al., 1998). Based on the study of the wheat–*Ae. speltoide* lines of different origin, it was demonstrated that the *Gc* genes are linked to genes determining resistance to leaf pathogens (Marais et al., 2010; Sibikeev et al., 2015; Sibikeev et al., 2016), which can be a serious problem for the widespread use of some of these lines for the production of commercial varieties (Marais et al., 2010).

In line 311/IR, unlike lines 311/134 and 311/FL, there is a very low level of development of R_0 plants forming seeds (see Table 3), which is probably associated with aneuploidy. This assumption may be consistent with the fact that among self-pollinated progenies of R_0 plants of line 311/IR there is a high incidence of aneuploids in R_1 (see Table 4).

The development of aneuploids among androgenic R_0 plants is associated not only with the presence of aneuploid gametes in the cultivated anthers from which embryos and then plants develop but also with the influence of the in vitro cultivation conditions (Oleszczuk et al., 2011). The variability of the chromosome number in androgenic R_0 common wheat plants and its hybrids is a typical phenomenon (Hu and Huang, 1987; Oleszczuk et al., 2011). For this reason, the selection of highly fertile 42-chromosome plants requires the formation of DH lines of the common wheat genotypes included in the selection. In this study R_0 seedlings were planted by clusters (families), and plants from one cluster may be genetically identical and genetically heterogeneous (Oleszczuk et al., 2014). In this regard, we isolated plants for the DH lines from R_1 generation based on the study of the chromosome number and formations of seeds per ear.

From the literature, it is known that the results of the cytological analysis and formation of seeds may serve as reliable indicators of aneuploidy in androgenic plants (Oleszczuk et al., 2011). Our data revealed that androgenic R_0 plants with a low level of seed formation in R_1 generation might not only be a source of aneuploidy but also of highly fertile 42-chromosome plants (see Table 4). Based on the example of lines 311/FL and 311/134, it was shown that the dihaploid lines formed based on such plants in the R_2 and R_3 generations remain fully fertile, and the selective cytogenetic analysis revealed the presence of plants with $2n = 42$.

In addition, note that R_0 plants with an increased fertility level (PF+, F, and FF groups) did not always stably manifested this trait when self-pollinating in the R_1 generation. Between the R_1 lines, formed from two R_0 regenerated plants (per208 and per317) of the parental line 311/134, differences in the manifestation of fertility and cytogenetic variability depending on the parental regenerant were found (see Table 5). Thus, all the studied reg208 lines in R_1 were represented mainly by plants with reduced fertility or sterility; however, the reg317 lines were represented by plants with increased fertility. The cytogenetic analysis revealed only 42-chromosome plants among plants of lines reg317, and aneuploids were found among plants of the reg208 lines. Based on the example of line 311/FL, it was also shown that R_0 plants with increased fertility in R_1 may show different levels of fertility and cytogenetic variability.

The 42-chromosome R_1 plants of lines reg208p2, reg317p2, and reg317p3 carrying the 1RS.1BL and 7DL-7Ai translocations, as well as the R_1 plant of the reg61p1 line with the 1RS.1BL translocation, characterized by full fertility (see Table 5), were used as the sources of the DH lines which retained a high fertility level during the breeding trials.

Thus, the obtained data showed the feasibility of selecting androgenic plants based on the expression of fertility and the chromosome number for the formation of dihaploid lines of the studied alloplasmic introgression genotypes of common wheat in R_1 generation. The prospects of using lines 311/134 and 311/FL in studies of the cultivation of anthers to produce more diverse dihaploid lines that are of interest for breeding were demonstrated.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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