Creation and study of emmer (*Triticum dicoccum*) × triticale hybrids

O.G. Silkova[1] ✉, Y.N. Ivanova[1], P.I. Stepochkin[2]

[1] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[2] Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics
   of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
✉ silkova@bionet.nsc.ru

Abstract. Triticale (× *Triticosecale* Wittmack) is of great interest as an insurance crop that can ensure the stability of the gross harvest of feed and food grains at a lower cost. In Western Siberia, only winter triticale varieties are cultivated, however, spring triticales are important for cultivation in regions not suitable for winter crops. To create spring varieties with high yields and good grain quality, it is necessary to study and enrich the gene pool, identify donors of economically valuable traits. One of the possible ways to solve this problem can be through the production of secondary hexaploid triticales with the involvement of the tetraploid wild-growing species of emmer wheat *Triticum dicoccum* (Schrank) Schuebl. The aim of this work was to create and study hybrids of emmer *T. dicoccum* (Schrank) Schuebl. with hexaploid triticale using genomic *in situ* hybridization for staining of meiotic chromosomes and analysis of plant productivity elements in F_4–F_8. DT4, DT5, DT6 plants and the prebreeding F_6 forms obtained from them – DT 4/168, DT 5/176 and DT 6/186 – were selected according to the characteristics of the productivity and the nature of the grain in the F_4 hybrid population. The offspring of hybrids DT4 and DT5 and prebreeding forms DT 4/168 and DT 5/176 had an increased grain nature (over 750 g/l), but low productivity. The hybrid DT6 and the breeding form DT 6/186 obtained from it had high grain productivity (785 ± 41 and 822 ± 74 g/m^2, respectively), but, like the paternal form of triticale UK 30/33, had a reduced nature of the grain. In F_8 DT 6/186 plants, 7 homologous pairs of rye chromosomes and from 27 to 30 wheat chromosomes were found in meiosis, which indicates the presence of a complete rye genome and two wheat AABB genomes. Rye chromosomes showed stable formation of bivalents in contrast to wheat chromosomes, which caused the presence of aneuploids in plant populations. Thus, hexaploid forms DT 4/168 and DT 5/176 with well-made smooth grain and high grain size were obtained, which can be used as a source of this trait for selection of food-grade triticale. DT 6/186 is a promising form for further breeding in order to obtain high-yielding forms of triticale.

Key words: triticale; *Triticum dicoccum*; wide hybrids; genomic *in situ* hybridization; productivity traits; meiosis; prebreeding forms.

Создание и изучение гибридов полба (*Triticum dicoccum*) × тритикале

О.Г. Силкова[1] ✉, Ю.Н. Иванова[1], П.И. Стёпочкин[2]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Сибирский научно-исследовательский институт растениеводства и селекции – филиал Федерального исследовательского центра
   Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
✉ silkova@bionet.nsc.ru

Аннотация. Тритикале (× *Triticosecale* Wittmack) представляет большой интерес как страховая культура, способная обеспечить стабильность валового сбора фуражного и продовольственного зерна с более низкими затратами. В Западной Сибири возделываются сорта только озимых тритикале, однако яровые тритикале являются значимыми для выращивания в регионах, не пригодных для озимых культур. Для создания яровых сортов с высокой урожайностью и хорошим качеством зерна необходимо изучение и обогащение генофонда, выделение доноров хозяйственно ценных признаков. Одним из возможных путей решения этой задачи может быть получение вторичных гексаплоидных тритикале с привлечением тетраплоидного дикорастущего вида пшеницы полба *Triticum dicoccum* (Schrank) Schuebl. Целью данной работы было создание и изучение гибридов полбы *T. dicoccum* (Schrank) Schuebl. с гексаплоидной тритикале с использованием геномной *in situ* гибридизации при окрашивании мейотических хромосом и анализ элементов продуктивности растений в F_4–F_8. По признакам продуктивности и натуры зерна в гибридной популяции F_4 были отобраны растения ДТ4, ДТ5, ДТ6

и выявленные в их потомстве пребридинговые формы F$_6$ – ДТ 4/168, ДТ 5/176 и ДТ 6/186. Потомства гибридов ДТ4 и ДТ5 и форм ДТ 4/168 и ДТ 5/176 имели повышенную натуру зерна (свыше 750 г/л), но невысокую продуктивность. Гибрид ДТ6 и полученная от него форма ДТ 6/186 отличались высокими показателями продуктивности зерна (785±41 и 822±74 г/м^2 соответственно), но, как и отцовская форма тритикале УК 30/33, имели пониженную натуру зерна. У растений потомства F$_8$ ДТ 6/186 в мейозе обнаружено 7 гомологичных пар хромосом ржи и от 27 до 30 хромосом пшеницы, что свидетельствует о наличии полного генома ржи и двух геномов пшеницы ААВВ. Хромосомы ржи демонстрировали стабильное формирование бивалентов в отличие от хромосом пшеницы, что вызвало анеуплоидию в популяциях растений. Таким образом, получены гексаплоидные формы ДТ 4/168 и ДТ 5/176 с хорошо выполненным гладким зерном и высокой натурой зерна, которые можно использовать в качестве источника этого признака для селекции тритикале пищевого направления. Форма ДТ 6/186 перспективна для дальнейшего селекционного процесса с целью получения высокоурожайных форм тритикале.

Ключевые слова: тритикале; *Triticum dicoccum*; отдаленные гибриды; геномная *in situ* гибридизация; признаки продуктивности; мейоз; пребридинговые формы.

Introduction

Triticale (×*Triticosecale* Wittmack) is a wheat and rye hybrid of a relatively short evolutionary history as an allopolyploid species. The first naturally created fertile wheat×rye hybrids were discovered in the late 1920s at the South-Eastern agricultural experimental station in Saratov (Meister, 1921). The plants had intermediate traits and were described as a new botanical species *Triticum Secalotriticum saratoviense* Meister by G.K. Meister (Levitsky, 1978). Meister immediately predicted the practical value of these intergeneric crossings. The first man-made wheat×rye hybrids (*Triticosecale* Wittmack) were obtained in 1888 by the German plant breeder W. Rimpau (Müntzing, 1974), who described 12 plants descending from a wheat×rye hybrid generally recognized as the first triticales (×*Triticosecale* Wittmack). The cytological analysis of the first triticales developed in Russia and Germany showed the somatic chromosome number of 56 (8x) (Müntzing, 1961; Levitsky, Benetzkaja, 1978), which demonstrated the combination of four genomes as follows: AABBDDRR, AABBDD, soft wheat, and RR rye.

Octoploid triticales were of great interest for plant breeders due to high seed set per spike, increased plant pathogen resistance, and resistance to environmental stresses. However, the primary triticale lines suffered from meiotic errors (Müntzing, 1961; Shkutina, Khvostova, 1971; Lukaszewski, Gustafson, 1987) and high frequency of aneuploidy in earlier generations (Krolow, 1962; Stepochkin, Vladimirov, 1978; Silkova et al., 2021), which caused reduced fertility. Grain shriveling and lateness of maturity in octoploid forms were also considered as limitations for their introduction as cultivars. As a result, there have been global efforts to develop the technology for obtaining more promising lines with various valuable breeding traits, which eventually produced hexaploid triticales (Shulyndin, 1975).

Primary hexaploid triticales of the AABBRR genome type were obtained as crossings of tetraploid wheats (*T. turgidum*, *T. durum*) and *S. cereale* rye (Müntzing, 1974). However, undesirable traits were not completely eliminated. Agronomically valuable traits were improved by enriching the triticale gene pool with crossing experiments involving octoploid lines and commercial soft wheat varieties, as well as hexaploid triticales with full rye genome and two wheat genomes

identified in the progeny of octoploid triticales as a result of D-genome chromosome elimination (Stepochkin, 1978; Dou et al., 2006; Zhou et al., 2012; Hao et al., 2013; Li et al., 2015; Evtushenko et al., 2019). The crossing of two primary octoploid triticales produced hexaploid offspring, and breeding programs were focused on hybridization of these octoploids with the hexaploids identified in the progeny (Pisarev, 1964; Jenkins, 1969; Ammar et al., 2004; Oettler, 2005). Hexaploid triticales were more stable in terms of productivity (Müntzing, 1974; Oettler, 2005). As a result, various recombinant forms of secondary triticales were derived and karyotyped (Merker, 1975; Gustafson, Bennett, 1976; Lukaszewski, Gustafson, 1983; Badaev et al., 1985; Cheng, Murata, 2002; Mergoum et al., 2009; Shishkina et al., 2009; Fu et al., 2014), and some of them having combined wheat-rye genomes showed commercial value (Merker, 1975; Oettler, 2005; Zhou et al., 2012).

Agronomic traits of triticales were further improved by intergeneric and interspecific crossings. *Aegilops crassa* (2n = 4x = 28; DDMcrMcr), *Ae. juvenalis* (2n = 6x = 42; DDCuCuMjMj), *Ae. squarrosa* (syn. *Ae. tauschii*; 2n = 2x = 14; DD) and *Ae. triaristata* (2n = 6x = 42; CuCuMtMtMt2Mt2) (Gruszecka et., 1996), *Agropyron intermedium* ssp. *trichophorum* (2n = 42) (Gupta, Fedak, 1986a), *Hordeum parodii* Covas (Gupta, Fedak, 1986b), *H. vulgare* L. (Balyan, Fedak, 1989) and *T. monococcum* L. (Neumann, Kison, 1992) plants were used in hybridization experiments with hexaploid triticales. Intergeneric polyploid triticales were also bred through hybridization with intermediate forms sharing at least one set of chromosomes (genome) with the triticale genome. These hybridization efforts produced plants with resistance genes against diseases. Lines isolated in the progeny of triticale hybrids (AABBRR) and amphidiploids (wheat × *Psathyrostachys huashanica*, 2n = 8x = 56, AABBDDNsNs) were resistant to yellow rust (Kang et al., 2016). Hybridization of a hexaploid triticale and an amphiploid intermediate form (*Ae. variabilis* × *S. cereale*, 2n = 6x = 42; UUSvSvRR) produced addition and substitution lines with the 3Sv *Ae. variabilis* chromosome carrying the powdery mildew resistance gene *Pm13* (Kwiatek et al., 2016). Addition and substitution lines for chromosome 2D with the leaf-rust resistance gene *Lr39* and semidwarf gene *Rht8*, as well as chromosome 3D (or 3D/3B) with the *Lr32*

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gene were isolated in the progeny of triticale × amphiploid hybrids (*Ae. tauschii* × *S. cereale*, $2n = 4x = 28$; DDRR) (Kwiatek et al., 2015).

Thanks to breeding achievements, triticale has become a new economically significant cereal species characterized by high grain and vegetative mass productivity, that could be used as forage and green feed (McGoverin et al., 2011; Ayalew et al., 2018). In the last three decades, its products have become increasingly significant, which is demonstrated by increasing crop acreage across the world, from 1,453,269 ha in 1994 to 4,157,018 ha in 2016. Triticale grains are used to produce bioethanol and food wrap, as well as various food products (bread, biscuits, pastas, flatbreads, and malt) (Zhu, 2018), and bran is used as the source of prebiotics and antioxidants for yoghurts. Food-grade triticale grains are comparable to wheat in macro- and micronutrient contents (Zhu, 2018). Protein content in triticale grains is 1–1.5 % higher than in wheat and 3–4 % higher than in rye, gluten content matches that in wheat or is 2–4 % higher (Meleshkina et al., 2015). However, triticale underperforms in test weight. This parameter is closely related to the plumpness and hardness of grains, as well as to their size and shape. Average test weight for wheat is 700–810 g/l. At test weights below 740 g/l, flour yield tends to decrease rapidly as test weight goes down. Most spring triticales have shriveling grains and low flour yields, which limits their use in bread making (Rakha et al., 2011).

Development of domestic high-productivity triticale varieties with high grain quality requires further study and enrichment of the gene pool, as well as identification of donors for economically valuable traits. One way to solve this problem is to obtain secondary hexaploid triticales using emmer wheat *T. dicoccum* (Schrank) Schuebl., a tetraploid wild-growing wheat with long, large, and plump grains.

The goal of the present study was to breed new forms of hexaploid triticales (AABBRR genome type) with improved test weights by crossing emmer (*T. dicoccum* Schrank, AABB genome) with triticale and study their productivity and meiotic stability with chromosome staining using genomic *in situ* hybridization.

Material and methods
Plant material. New forms of hexaploid triticale were obtained by hybridization of emmer (*T. dicoccum* (Schrank) Schuebl.) and triticale (×*Triticosecale* Wittmack). Intergeneric F_1 hybrid of emmer lines (L133 × PKK) × k-25516 (AABB genome) was used as maternal plants. Awned semi-hulless emmer (L133 × PKK) created by VIR researchers is characterized by brittle spikes and low productivity, and awnless emmer k-25516 was obtained at Siberian Research Institute of Plant Production and Breeding (SRI PPB), ICG SB RAS from the population of awned emmer wheat from the VIR collection. The paternal plants were represented by a selection form of hexaploid triticale UK 30/33 selected from the population of cytogenetically unstable octoploid triticale UK30 (AABBDDRR genome) developed at the SRI PPB by crossing the Ulyanovka soft wheat variety with the Korotkostebelnaya 69 short stem rye with subsequent doubling of chromosome number induced by colchicine water solution.

The progeny of three F_4 hybrids DT4, DT5, and DT6, and three selection forms DT 4/168, DT 5/176, and DT 6/186 isolated in F_5 from hybrid populations DT4, DT5, and DT6 respectively was selected for the study. In 2020, their F_6 progeny was seeded for research purposes along with F_4 hybrids at the nursery for distant wheat hybrids in the field of SRI PPB.

Fluorescence *in situ* hybridization of meiotic chromosomes. To evaluate the meiotic stability in the selection forms, two most productive plants DT 6/186/156 and DT 6/186/165 were selected in the F_7 progeny of plant DT 6/186, and their F_8 seeds were seeded in the hydroponic greenhouse of the ICG SB RAS in spring 2021. The chromosomal behavior of the progeny was studied using routine acetocarmine staining protocol and FISH staining (fluorescence *in situ* hybridization) following the technique described earlier (Ivanova et al., 2019).

The meiocytes were analyzed at the diakinesis stage, metaphase I (MI), anaphase I (AI), and telophase II (TII). The probes used in the analysis were as follows: *Aegilops tauschii* pAet6-09 specific for centromere repeats in rice, wheat, rye, and barley chromosomes (Zhang et al., 2004); pAWRc specific for centromere repeats in rye chromosomes (Francki, 2001), and rye genomic DNA. DNA repeat samples of pAet6-09 and pAWRc were the courtesy of Dr. A. Lukaszewcki (University of California, Riverside, the United States). Centromere-specific probes were labeled with biotin 16-dUTP or digoxigenin 11-dUTP by means of polymerase chain reaction (PCR). The total rye DNA was labeled by Nick translation with digoxigenin 11-dUTP. The probes were combined in different proportions and mixed with blocking wheat DNA. The preparations were mounted in Vectashield antifade solution (Vector Laboratories) slowing down fluorescence fading and including 1 µg/ml DAPI (4′,6-diamidino-2-phenylindol, Sigma-Aldrich, the United States) for chromatin staining. All preparations were analyzed using an Axio Imager M1 microscope (Karl Zeiss, Germany), the images were recorded using a ProgRes MF camera (Meta Systems, Jenoptic) at the Center of Microscopic Analysis of Biological Objects, SB RAS and processed using Adobe Photoshop CS2 software.

Analysis of economically valuable traits. Structural analysis of the plants was performed at the facility equipped for metric measurements, threshing, and seed weighting. As a result, the following data on productivity elements were obtained: spike length; spike density; grain weight per spike; thousand kernel weight; grain number per spikelet; test weight measured using a microchondrometer (Stepochkina, Stepochkin, 2015); and grain productivity per 1 m². Statistical processing of the results was carried out following the standard technique (Dospekhov, 1985). Significance of differences between mean values of two samples was estimated using Student's *t*-test.

Results
The parental-line plants had different spike and grain morphology. The emmer plants (L133 × PKK) had short, awned, brittle spikes and smooth, long grains (Fig. 1, *a*). The k-25516 emmer sample was an awnless plant with thin, long grains (see Fig. 1, *b*). Triticale UK 30/33 had a dense, awned spike and

Fig. 1. Spikes and seeds: *a*, emmer wheat (L133×PKK); *b*, emmer wheat k-25516; *c*, triticale UK 30/33; F_4 hybrids: *d*, DT4; *e*, DT6; *f*, DT5.

Table 1. Results of structural analysis of triticale plants and emmer × triticale hybrids

Name of triticale line or emmer × triticale hybrid	Spike length, cm	Spike density	Grain weight per spike, g	Thousand kernel weight, g	Grain number per spikelet	Test weight, g/l	Productivity, g/m²
UK 30/33	8.3±0.3	2.89±0.08	2.6±0.2	47±2	2.48±0.10	706±8	584±33
DT4	11.0±0.4**	2.10±0.06	2.1±0.2	50±2	1.78±0.10**	806±14***	435±34*
DT6	10.2±0.2**	3.14±0.07*	3.7±0.2**	48±1	2.43±0.09	749±16**	785±41**
DT5	8.3±0.1	2.64±0.12*	1.3±0.2*	39±3*	1.56±0.11**	789±7***	280±44**
DT 4/168	10.8±0.8**	2.31±0.08**	2.5±0.4	47±2	2.15±0.26	760±20*	531±92
DT 6/186	10.8±0.5**	2.38±0.14**	3.9±0.3**	48±5	3.20±0.18**	706±25	822±74**
DT 5/176	9.6±0.7*	2.28±0.11**	2.0±0.3	47±3	1.95±0.13**	772±48*	423±67*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ – significant differences between the hybrid and 6x triticale UK 30/33.

grains similar to those of soft wheat in shape but shriveling (see Fig. 1, *c*).

Plants of three emmer×triticale F_4 hybrids had different spike morphology (Table 1, see Fig. 1, *d–f*). The DT6 hybrid had a dense, awned spike. The plants of the remaining two hybrids had looser spikes. The spikes were sterile at the ends and therefore often susceptible to ergot.

All hybrids had a hair neck similar to the paternal triticale UK 30/33. This means that these genotypes have a gene re-sponsible for manifestation of this trait localized in the long arm of chromosome 5R.

Triticale UK 30/33 had a short spike, low test weight, and medium grain productivity (see Table 1). Selection forms and hybrids, except for DT5, had a longer spike than the paternal form UK 30/33. Hybrid DT5 had low productivity due to flower sterility in the upper part of the spike and low thousand kernel weight. Hybrid DT4 had a high thousand kernel weight of 50±2 g, and the highest test weight of 806±14 g/l.

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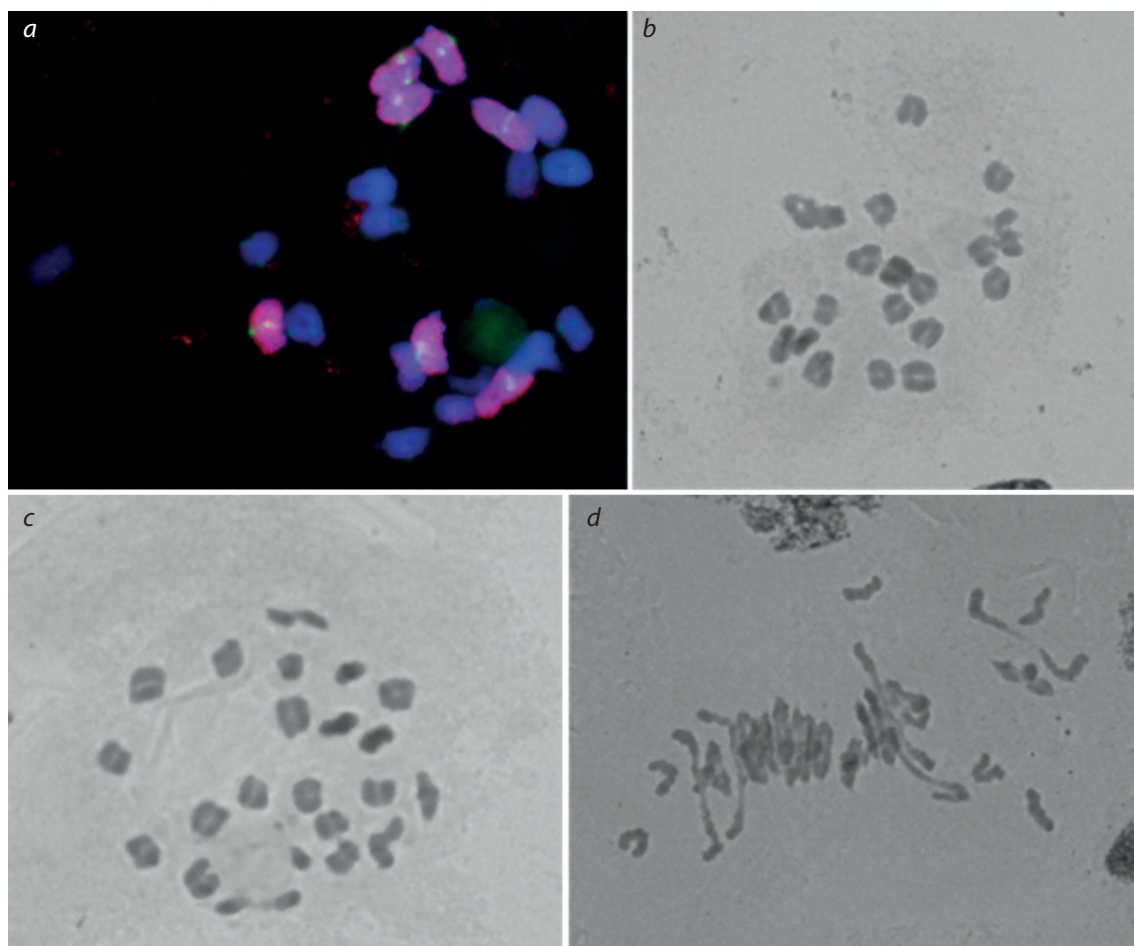полба (*Triticum dicoccum*) × тритикале

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Fig. 2. Diakinesis (*a–c*) and metaphase I (*d*) meiotic stages in the progeny of DT 6/186/156 plant (*a, b*) and DT 6/186/165 plant (*c, d*).

a, b, 21 bivalents, *a*, 7 rye bivalents; *c*, 21 bivalents and one univalent; *d*, univalents in metaphase I. *a*, genomic *in situ* hybridization, rye chromosomes stained red; *b–d*, acetocarmine staining.

However, its grain productivity, although higher than that of DT5, was still not too high due to low seed set of spikes and spikelets. Flowers at the top of the spike were often sterile as well. Hybrid DT6 was characterized by dense spikes and high grain productivity (785 ± 41 g/m²), as well as seed set of spikes and spikelets. The spikes were fertile along their full length.

Selection forms DT 4/168, DT 5/176, and DT 6/186 had different grain weights per spike, grain numbers per spike, test weights, and productivities. DT 4/168 had denser spikes and slightly higher seed sets of spikes and spikelets compared to the DT4 hybrid it was obtained from. Breeding sample DT 5/176 was more productive than the initial DT5 hybrid, but it had the lowest productivity among the three breeding samples studied. Small spike size and sterility of 3–7 spikelets in the upper part of the spike resulted in low productivity of hybrids DT5 and DT 5/176. The DT 6/186 line had the highest seed set and productivity among the studied lines. Despite the lowest test weight values similar to triticale UK 30/33 (706 ± 25 g/l), grain productivity per plot reached 822 ± 74 g/m². DT 6/186 had a smoother overall morphology than the initial hybrid DT6, but higher breeding value due to its higher grain productivity.

Cytological analysis of the progeny of two plants (DT 6/186/156 and DT 6/186/165) from the highly productive DT 6/186 line revealed instability in chromosome number and errors in chromosomal behavior in the first and second meiotic divisions. Chromosome staining using genomic *in situ* hybridization in these plants showed 14 rye chromosomes forming bivalents, which implied the presence of seven homologous pairs (Fig. 2, *a*). However, the bivalent chromosomes demonstrated premature separation in some meiocytes (desynapsis), with rye chromosomes becoming univalent and distributing anomalously between the poles (Fig. 3, *a*).

Chromosome number in the discovered aneuploid plants varied from $2n = 41$ to $2n = 44$. Among the ten plants from the DT 6/186/156 progeny, only one plant had chromosome number $2n = 42$ (see Fig. 2, *b*), while there were no plants with euploid chromosome numbers in the progeny of DT 6/186/165.

Univalents were discovered in the metaphase I (see Fig. 2, *d*), which were lagging at the equator during chromosome separation in anaphase I in 86.75 ± 4.56 and 61.32 ± 2.81 % of cells in DT 6/186/156 and DT 6/186/165 respectively (Table 2, see Fig. 3, *c, d*). The lagging chromosomes were divided into sister chromatids (see Fig. 3, *a, c, d*)
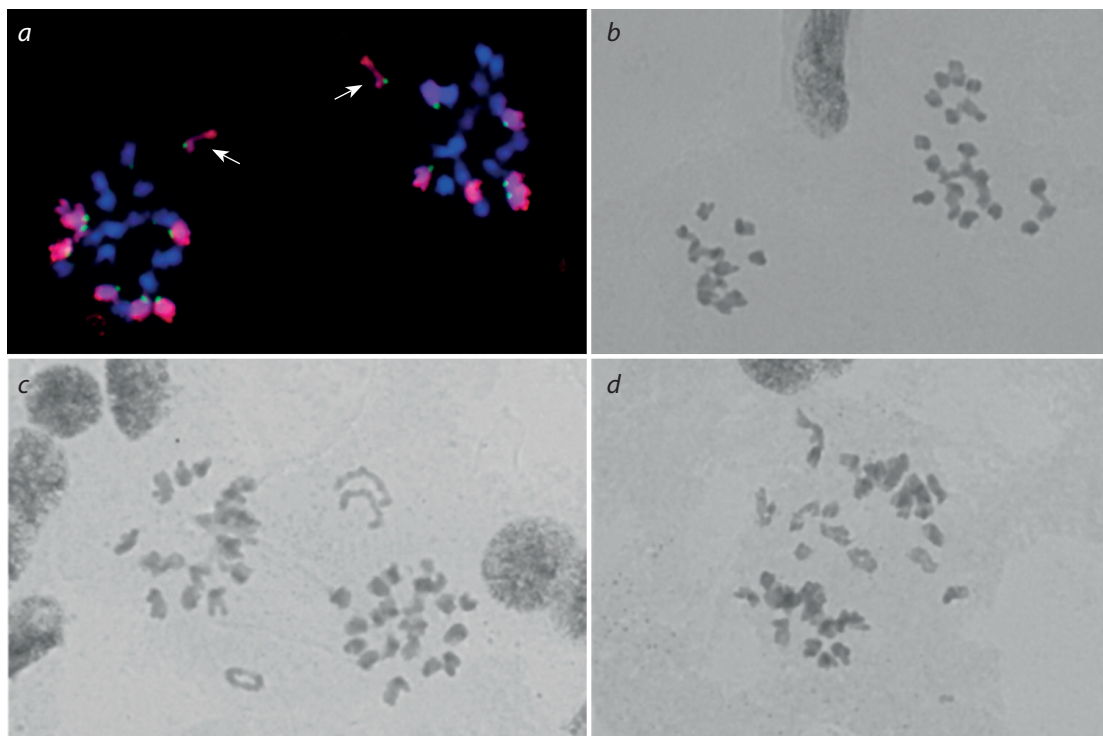
Fig. 3. Chromosome separation anomalies in meiotic anaphase I in the progeny of DT 6/186/165: *a*, unequal separation of wheat and rye chromosomes, the rye chromosome is divided into sister chromatids (arrows); *b*, unequal chromosome separation; *c*, *d*, lagging chromosomes at the equator and univalent division into sister chromatids.

a, genomic *in situ* hybridization, rye chromosomes stained red; *b–d*, acetocarmine staining.
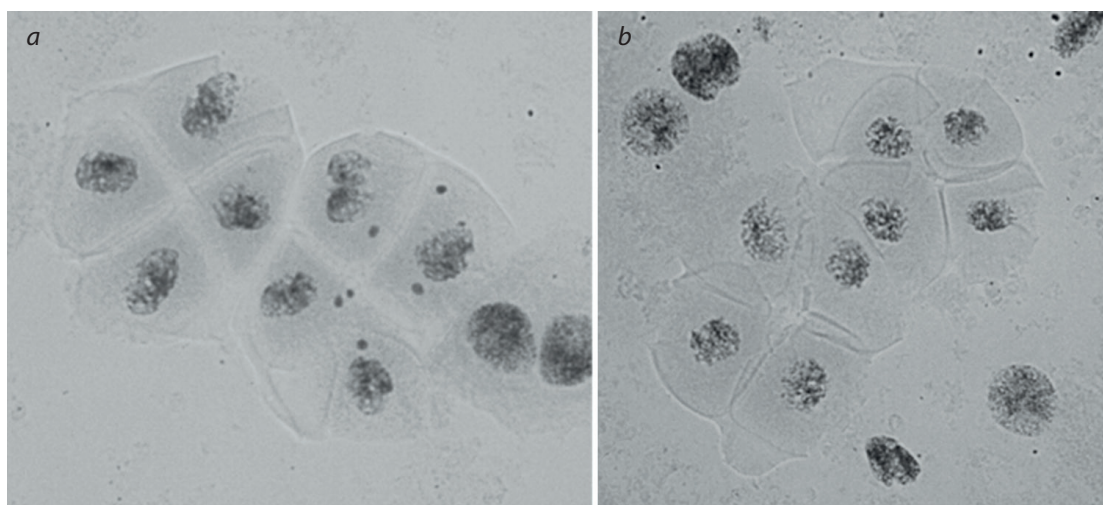

Fig. 4. Tetrads with (*a*) and without (*b*) micronuclei.

or broken at the centromere. Instability of chromosome separation during the division resulted in micronuclei formation at the tetrad stage (Fig. 4, *a*).

Micronuclei were observed in 60.29 ± 3.14 and 72.16 ± 2.29 % tetrads in DT 6/186/156 and DT 6/186/165, respectively (see Table 2). Even the euploid plant with $2n = 42$ (DT 6/186/156) had micronuclei in 51.48 % tetrads, which prevented us from considering this plant fully cytogenetically stable.

Plants with the minimum number of anomalous meiocytes in anaphase I and telophase II were selected from populations DT 6/186/156 and DT 6/186/165 based on the results of the analysis.

Discussion

Triticale (× *Triticosecale* Wittmack) as an agricultural crop combines wheat's high yield potential with rye's resistance to biotic and abiotic stresses, which increases its adaptability

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Table 2. Analysis of meiotic chromosomal behavior in F_8 plants

Selection form	Total analyzed in anaphase I		Number of meiocytes with anomalous chromosome separation in anaphase I, %	Total analyzed by tetrads		Number of tetrads with micronuclei, %
	plants	cells		plants	cells	
DT 6/186/156	10	534	86.75 ± 4.56	8	1830	60.29 ± 3.14
DT 6/186/165	9	698	61.32 ± 2.81	8	2866	72.16 ± 2.29

to cultivation conditions in salty or highly acidic soils and in presence of toxic heavy metals. Thanks to these traits, triticale is of great interest as an emergency crop ensuring stable gross harvest of forage and food grains at lower costs (McGoverin et al., 2011). Despite today's applications of triticale grains being mostly restricted to forage in animal husbandry and production of feed and bioethanol, there is a rising interest in the use of triticale grains in human food products. Triticale grains have proven nutritional and dietary value (Meleshkina et al., 2015; Zhu, 2018), since they include not only proteins, carbohydrates, and fats, but also vitamins, minerals, and dietary fibres (14–18 %) (Rakha et al., 2011; Zhu, 2018). Compared to that of wheat, the protein from its grains has a richer amino acid profile, particularly in indispensable amino acids, such as lysine, threonine, and leucine (Meleshkina et al., 2015; Torikov et al., 2019). Triticale starch amounting to 3/4 of the kernel weight has a significantly lower amylose content compared to rye and wheat (Zhu, 2018), which ensures its better digestion by humans (Meleshkina et al., 2015).

To increase the share of triticales in production of breads and pastries, in recent decades breeding efforts have been aimed at increasing the quality of grains and finished products, which has resulted in development of triticale bread-making varieties (Grabovets et al., 2013), so State standards for triticale flour have been developed (State Standard 34142-2017). The characteristics of winter triticale varieties also include applicability for bread making. Triticale breeding efforts in Russia and other countries are primarily aimed at developing winter varieties (State Register…, 2022). However, grain quality assessment of the samples from the spring triticale collection showed that spring triticales have good potential for creating bread-making varieties (Krokhmal, Grabovets, 2015; Bocharnikova et al., 2017; Abdelkawy et al., 2020; Yerzhebayeva et al., 2020). The samples with such improved parameters as protein content, test weight, falling number, vitreousness, gluten quantity and quality, etc., are used in breeding for improved grain quality (Krokhmal, Grabovets, 2015; Bocharnikova et al., 2017; Turbayev et al., 2019; Abdelkawy et al., 2020; Yerzhebayeva et al., 2020).

A trait such as high test weight can be transferred by distant hybridization. This parameter is closely related to genetically determined parameters such as grain plumpness, hardness, and shape. In the present paper, tetraploid emmer wheat *T. dicoccum* (Schrank) Schuebl. with long, large, and plump grains was used as the maternal line for hybridization with hexaploid triticale (× *Triticosecale* Wittmack). As a result, new hexaploid forms of triticale with AABBRR genome types were created,

which is confirmed by the analysis of meiotic chromosomal behavior using genomic *in situ* hybridization. Seven pairs of rye chromosomes and 27 to 30 wheat chromosomes were observed in the plants, which implies the presence of a complete rye genome and two wheat genomes. Three plants, DT4, DT5, and DT6, were isolated in the F_4 population, the progeny of which demonstrated test weight values significantly exceeding those of the initial triticale line, and test weights in the progeny of DT 4/168, DT 5/176, and DT 6/186 forms from F_6 were higher or on par with those of the initial triticale line. These plants had different productivity values, with the highest ones in F_4 recorded in the DT6 line (785 ± 41 g/m^2). The respective value for DT 6/186, i. e., the progeny of the latter in F_6, reached 822 ± 74 g/m^2. The DT 4/168 and DT 5/176 lines are of moderate interest for further research into improvement of bread-making properties of triticale grains.

The study of the meiotic behavior of rye and wheat chromosomes in the progeny of F_8 plants DT 6/186/156 and DT 6/186/165 showed that they had not yet achieved cytogenetic stability, which was indicated by the discovered chromosome separation errors and the presence of aneuploids in the populations. Chromosome separation errors mostly occurred in wheat chromosomes due to their monosomy. Cytological instability and aneuploidy in octoploid and hexaploid wheat-rye allopolyploids have been an issue from the start (Shkutina, Khvostova, 1971; Kaltsikes, 1974; Weimarck, 1974; Lukaszewski, Gustafson, 1987), but secondary triticales turned out to be more cytogenetically stable than primary ones (Kaltsikes, 1974). Cytological study of triticales showed that the interaction between wheat and rye genomes in the cells of the same plant resulted in physiological defects in cells persisting for decades at least. Meiotic and mitotic errors were found both in the triticale obtained by Rimpau in 1888 (Levitsky, 1978; Müntzing, 1974) and in the triticales obtained later. Despite the complete set of chromosomes, meiotic univalents were found in triticales with different ploidy levels (Shkutina, Khvostova, 1971; Kaltsikes, 1974; Lukaszewski, Gustafson, 1987; Olesczuk, Lukaszewski, 2014; Orlovskaya et al., 2015). The study into meiotic chromosomal behavior in triticales in the present and earlier papers demonstrated that while only bivalents were present at the diakinesis stage, univalents appeared in metaphase I as a result of to produce lagging chromosomes at the equator (Shkutina, Khvostova, 1971). Presumably, aneuploid cells in triticales may emerge as a result of asynchronous separation of rye and wheat chromosomes and their lagging in anaphase and telophase (Shkutina, Khvostova, 1971). The presumed cause of meiotic instability in the obtained am-

phidiploids is an imbalance in the genetic system of meiotic pairing and the differences in cell cycles between wheat and rye (Müntzing, 1974).

It is known for a fact that chromosome separation depends on kinetochore function (Sanei et al., 2011). It was found that kinetochore protein CENH3 produced by one of the parents maintained the function of other parental kinetochores in stable hybrids, despite the differences between DNA sequences of centromere regions in parental lines (Ishii et al., 2016). It was also shown that cytogenetic stability in triticales could also be linked to increased expression of rye-specific CENH3 forms in a hybrid genome (Evtushenko et al., 2019).

Another possible cause of cytogenetic instability of the obtained triticales could be a nuclear-cytoplasmic incompatibility, because the hybrids are bred using an intergeneric F_1 hybrid of emmer lines (*T. dicoccum* (Schrank) Schuebl.) (L133 × PKK) × k-25516 (genome AABB) as the maternal form. The AABB genomes in paternal triticale forms originate from soft wheat, while the rye genome is used as the base. Selection of genotypes of wheat varieties during triticale backcrossing may lead to recovery of fertility and cytogenetic stability in new forms, as exemplified by alloplasmic soft wheat lines (*H. vulgare*)-*T. aestivum* (Pershina et al., 1999, 2018; Trubacheeva et al., 2021).

Conclusion

The result of this work was to create and study hybrids of emmer *T. dicoccum* (Schrank) Schuebl. with hexaploid triticale. According to the characteristics of productivity and test weight values of the grain, hexaploid prebreeding forms F_6 were isolated – DT 4/168, DT 5/176 and DT 6/186. Thus, hexaploid forms DT 4/168 and DT 5/176 with well-filled, smooth grains and high test weights that can be used as the source of said traits in food-grade triticale breeding have been obtained. The DT 6/186 line shows promise in terms of breeding for high yields. As a result of the analysis of meiotic division in DT 6/186 plants, this sample is unstable for wheat chromosomes, therefore, aneuploids will occur in the offspring. To restore fertility and cytogenetic stability of new forms of triticale with emmer cytoplasm and chromosomes, it is necessary to select the genotypes of wheat varieties during backcrossing.

References

Abdelkawy R.N.F., Turbayev A.Zh., Solov'iev A.A. Technological properties of spring triticale grain grown in the Central Economic Region, Russia. *Vestnik Kurskoy Gosudarstvennoy Selskokhozyastvennoy Akademii = Bulletin of the Kursk State Agricultural Academy.* 2020;5:87-97. (in Russian)

Ammar K., Mergoum M., Rajaram S. The history and evolution of triticale. In: Triticale Improvement and Production (FAO Plant Production and Protection Paper. 179). Rome, 2004;1-10.

Ayalew H., Kumssa T.T., Butler T.J., Ma X.-F. Triticale improvement for forage and cover crop uses in the Southern Great Plains of the United States. *Front. Plant Sci.* 2018;9:1130. DOI 10.3389/fpls.2018.01130.

Badaev N.S., Badaeva E.D., Bolsheva N.L., Maximov N.G., Zelenin A.V. Cytogenetic analysis of forms produced by crossing hexaploid triticale with common wheat. *Theor. Appl. Genet.* 1985;70(5):536-541. DOI 10.1007/BF00305987.

Balyan H.S., Fedak G. Meiotic study of hybrids between barley (*Hordeum vulgare* L.) and Triticale (× *Triticosecale* Wittmack). *J. Hered.* 1989;80(6):460-463. DOI 10.1093/oxfordjournals.jhered.a110898.

Bocharnikova O.G., Gorbunov V.N., Shevchenko V.E. Assessment of spring triticale varieties in performance and grain quality. *Vestnik Voronezhskogo Gosudarstvennogo Agrarnogo Universita = Vestnik of Voronezh State Agrarian University.* 2017;2(53):23-30. DOI 10.17238/issn2071-2243.2017.2.23. (in Russian)

Cheng Z.J., Murata M. Loss of chromosomes 2R and 5RS in octoploid triticale selected for agronomic traits. *Genes Genet. Syst.* 2002;77(1):23-29. DOI 10.1266/ggs.77.23.

Dospekhov B.A. Methodology of Field Experiments. Moscow: Agropromizdat Publ., 1985. (in Russian)

Dou Q., Tanaka H., Nakata N., Tsujimoto H. Molecular cytogenetic analyses of hexaploid lines spontaneously appearing in octoploid Triticale. *Theor. Appl. Genet.* 2006;114(1):41-47. DOI 10.1007/s00122-006-0408-x.

Evtushenko E.V., Lipikhina Y.A., Stepochkin P.I., Vershinin A.V. Cytogenetic and molecular characteristics of rye genome in octoploid triticale (× *Triticosecale* Wittmack). *Comp. Cytogenet.* 2019;13(4):423-434. DOI 10.3897/CompCytogen.v13i4.39576.

Francki M.G. Identification of Bilby, a diverged centromeric T1-copia retrotransposon family from cereal rye (*Secale cereale* L.). *Genome.* 2001;44(2):266-274. DOI 10.1139/g00-112.

Fu S., Ren Z., Chen X., Yan B., Tan F., Fu T., Tang Z. New wheat-rye 5DS-4RS·4RL and 4RS-5DS·5DL translocation lines with powdery mildew resistance. *J. Plant Res.* 2014;127(6):743-753. DOI 10.1007/s10265-014-0659-6.

Grabovets A.I., Krokhmal A.V., Dremucheva G.F., Karchevskaya O.E. Breeding of triticale for baking purposes. *Russian Agricultural Sciences.* 2013;39(3):197-202.

Gruszecka D., Tarkowski C., Stefanowska G., Marciniak K. Cytological analysis of F₁ Aegilops hybrids with Triticosecale. In: Guedes-Pinto H., Darvey N., Carnide V.P. (Eds.) Triticale: Today and Tomorrow. Developments in Plant Breeding. Vol. 5. Dordrecht: Springer, 1996;195-201. DOI 10.1007/978-94-009-0329-6_24.

Gupta P.K., Fedak G. Intergeneric hybrids between × *Triticosecale* cv. Welsh (2*n* = 42) and three genotypes of *Agropyron intermedium* (2*n* = 42). *Can. J. Genet. Cytol.* 1986a;28(2):176-179. DOI 10.1139/g86-024.

Gupta P.K., Fedak G. Variation in induction of homoeologous pairing among chromosomes of 6*x Hordeum parodii* as a result of three triticale (× *Triticosecale* Wittmack) cultivars. *Can. J. Genet. Cytol.* 1986b;28(3):420-425. DOI 10.1139/g86-062.

Gustafson J.P., Bennett M.D. Preferential selection for wheat-rye substitutions in 42-chromosome triticale. *Crop Sci.* 1976;16(5):688-693. DOI 10.2135/cropsci1976.0011183X001600050022x.

Hao M., Luo J., Zhang L., Yuan Z., Yang Y., Wu M., Chen W., Zheng Y., Zhang H., Liu D. Production of hexaploid triticale by a synthetic hexaploid wheat-rye hybrid method. *Euphytica.* 2013;193:347-357. DOI 10.1007/s10681-013-0930-2.

Ishii T., Karimi-Ashtiyani R., Houben A. Haploidization via chromosome elimination: means and mechanisms. *Annu. Rev. Plant Biol.* 2016;67:421-438. DOI 10.1146/annurev-arplant-043014-114714.

Ivanova Yu.N., Solovey L.A., Loginova D.B., Miroshnikova E.E., Dubovets N.I., Silkova O.G. The creation and characterization of the bread wheat line with a centric translocation T2DL.2RL. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding.* 2019;23(7):846-855. DOI 10.18699/VJ19.558. (in Russian)

Jenkins B.C. History of the development of some presently promising hexaploid *Triticales*. *Wheat Inform. Serv.* 1969;28:18-20.

Kaltsikes P.J. Univalency in triticale. In: Macintyre R., Campbell M. (Eds.) Triticale: Proceedings of an international symposium. El Batan, Mexico: Int. Develop. Res. Centre, 1974;159-167.

Kang H., Wang H., Huang J., Wang Y., Li D., Diao C., Zhu W., Tang Y., Wang Y., Fan X., Zeng J., Xu L., Sha L., Zhang H., Zhou Y. Di-

О.Г. Силкова, Ю.Н. Иванова
П.И. Стёпочкин

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полба (*Triticum dicoccum*) × тритикале

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vergent development of hexaploid triticale by a wheat–rye–*Psathy-rostachys huashanica* trigeneric hybrid method. *PloS One*. 2016; 11(5):e0155667. DOI 10.1371/journal.pone.0155667.

Krokhmal A.V., Grabovets A.I. Triticale grain quality formation. *Izves-tiya Orenburgskogo Gosudarstvennogo Agrarnogo Universiteta = Proceedings of the Orenburg State Agrarian University.* 2015;2(52): 46-48. (in Russian)

Krolow K.D. Aneuploidie und Fertilitat bei amphidiploiden Weizen-Roggen-Bastarden (*Triticale*). I. Aneuploidie un Selection auf Fertili-tät bei oktoploiden Triticale-Formen. *Z. Pflanzenzucht*. 1962;48(2): 177-196.

Kwiatek M., Belter J., Majka M., Wiśniewska H. Allocation of the S-genome chromosomes of *Aegilops variabilis* Eig. carrying pow-dery mildew resistance in triticale (× *Triticosecale* Wittmack). *Pro-toplasma*. 2016;253:329-343. DOI 10.1007/s00709-015-0813-6.

Kwiatek M., Majka M., Wiśniewska H., Apolinarska B., Belter J. Ef-fective transfer of chromosomes carrying leaf rust resistance genes from *Aegilops tauschii* Coss. into hexaploid triticale (× *Triticosecale* Witt.) using *Ae. tauschii* × *Secale cereale* amphiploid forms. *J. Appl. Genet*. 2015;56:163-168. DOI 10.1007/s13353-014-0264-3.

Levitsky G.A. On the history of prolific intermediate constant wheat-rye hybrids. In: Levitsky G.A. Plant Cytogenetics. Moscow: Nauka Publ., 1978;251-253. (in Russian)

Levitsky G.A., Benetzkaja G.K. Cytology of the wheat-rye amphidi-ploids. In: Levitsky G.A. Plant Cytogenetics. Moscow: Nauka Publ., 1978;224-250. (in Russian)

Li H., Guo X., Wang C., Ji W. Spontaneous and divergent hexaploid triticales derived from common wheat × rye by complete elimination of D-genome chromosomes. *PLoS One*. 2015;10(3):e0120421. DOI 10.1371/journal.pone.0120421.

Lukaszewski A.J., Gustafson J.P. Translocations and modifications of chromosomes in triticale × wheat hybrids. *Theor. Appl. Genet.* 1983; 63(1):49-55. DOI 10.1007/BF00303771.

Lukaszewski A.J., Gustafson J.P. Cytogenetics of triticale. In: Janick J. (Ed.) Plant Breeding Reviews. 1987;5:41-93. DOI 10.1002/978111 8061022.ch3.

McGoverin C.M., Snyders F., Muller N., Botes W., Fox G., Manley M. A review of triticale uses and the effect of growth environment on grain quality. *J. Sci. Food Agric.* 2011;91(7):1155-1165. DOI 10.1002/jsfa.4338.

Meister G.K. Natural hybridization of wheat and rye in Russia. *J. Hered.* 1921;12(10):467-470. DOI 10.1093/oxfordjournals.jhered. a102049.

Meleshkina E.P., Pankratieva I.A., Politukha O.V., Chirkova L.V., Zhil-tsova N.S. Evaluation of triticale grain quality. *Khleboprodukty = Grain Products.* 2015;2:48-49. (in Russian)

Mergoum M., Singh P.K., Peña R.J., Lozano-del Río A.J., Cooper K.V., Salmon D.F., Gómez-Macpherson H. Triticale: a "new" crop with old challenges. In: Carena M. (Ed.) Cereals. Handbook of Plant Breeding. Vol. 3. New York: Springer, 2009. DOI 10.1007/978-0-387-72297-9_9.

Merker A. Chromosome composition of hexaploid triticale. *Hereditas*. 1975;80(1):41-52. DOI 10.1111/j.1601-5223.1975.tb01498.x.

Müntzing A. Genetic Research. Stockholm: Lts Förlag, 1961. (Russ. ed.: Muentzing A. Geneticheskie Issledovaniya. Moscow, 1963. (in Russian)

Müntzing A. Historical review of the development of triticale. In: Macintyre R., Campbell M. (Eds.) Triticale: Proceedings of an in-ternational symposium. El Batan, Mexico, 1–3 October 1973. Int. Develop. Res. Centre, 1974;13-30.

Neumann M., Kison H.U. Hybridization between hexaploid triti-cale and *Triticum monococcum* L. II. The F_1 generation A^uA^bBR. *Hereditas*. 1992;116(3):291-294. DOI 10.1111/j.1601-5223.1992. tb00157.x.

Oettler G. The fortune of a botanical curiosity – Triticale: past, present and future. *J. Agric. Sci.* 2005;143(5):329-346. DOI 10.1017/S002 1859605005290.

Olesczuk S., Lukaszewski A.J. The origin of unusual chromosome con-stitutions among newly formed allopolyploids. *Am. J. Bot.* 2014; 101(2):318-326. DOI 10.3732/ajb.1300286.

Orlovskaya O.A., Leonova I.N., Adonina I.G., Salina E.A., Khoty-leva L.V., Shumny V.K. Molecular cytogenetic analysis of triticale and wheat lines with introgressions of the genetic material of Tri-ticeae tribe species. *Russ. J. Genet. Appl. Res.* 2016;6(5):527-536. DOI 10.1134/S2079059716050087.

Pershina L.A., Belova L.I., Trubacheeva N.V., Osadchaya T.S., Sh-umny V.K., Belan I.A., Rosseeva L.P., Nemchenko V.V., Abaku-mov S.N. Alloplasmic recombinant lines (*H. vulgare*)-*T. aestivum* with 1RS.1BL translocation: initial genotypes for production of common wheat varieties. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding.* 2018;22(5):544-552. DOI 10.18699/VJ18.393.

Pershina L.A., Numerova O.M., Belova L.I., Devyatkina E.P., Shum-ny V.K. Restoration of fertility in backcross progeny of barley-wheat hybrids *Hordeum vulgare* L. ($2n = 14$) × *Triticum aestivum* L. ($2n = 42$) in relation to wheat genotypes involved in backcrosses. *Russ. J. Genet.* 1999;35(2):176-183.

Pisarev V.E. Breeding of Grain Crops. Moscow, 1964. (in Russian)

Rakha A., Åman P., Andersson R. Dietary fiber in triticale grain: varia-tion in content, composition, and molecular weight distribution of extractable components. *J. Cereal Sci.* 2011;54(3):324-331. DOI 10.1016/j.jcs.2011.06.010.

Sanei M., Pickering R., Kumke K., Nasuda S., Houben A. Loss of centromeric histone H3 (CENH3) from centromeres precedes uni-parental chromosome elimination in interspecific barley hybrids. *Proc. Natl. Acad. Sci. USA.* 2011;108(33):E498-E505. DOI 10.1073/ pnas.1103190108.

Shishkina A.A., Badaeva E.D., Soloviev A.A. Features of karyotype organization in some forms of spring triticale. *Izvestiya Timiryazev-skoy Selskokhozyaystvennoy Akademii = Proceedings of the Timi-ryazev Agricultural Academy*. 2009;2:123-130. (in Russian)

Shkutina F.M., Khvostova V.V. Cytological investigation of *Triti-cale. Theor. Appl. Genet.* 1971;41:109-119. DOI 10.1007/BF002 77752.

Shulyndin A.F. Genetic grounds for the synthesis of various triticales and their improvement by breeding. In: Triticale: Study and Breed-ing. Leningrad: VIR Publ., 1975;53-56. (in Russian)

Silkova O.G., Ivanova Y.N., Loginova D.B., Solovey L.A., Syche-va E.A., Dubovets N.I. Karyotype reorganization in wheat–rye hy-brids obtained via unreduced gametes: is there a limit to the chromo-some number in triticale? *Plants.* 2021;10(10):2052. DOI 10.3390/ plants10102052.

State Register for Selection Achievements Admitted for Usage (Na-tional List). Vol. 1. Plant varieties (official publication). Moscow: Rosinformagrotech, 2022. (in Russian)

State Standard 34142-2017. Tritical Flour: Specifications. 2018. Avail-able at: https://docs.cntd.ru/document/1200146040 (in Russian)

Stepochkin P.I. The appearance of 6*x* triticale plants among the C_2 offspring of homogenomic 8*x* triticale. *Genetika = Genetics (Mos-cow)*.1978;14(9):1658-1659. (in Russian)

Stepochkin P.I., Vladimirov N.S. Chromosome number, seed set, and winter hardiness in C_1 winter lines of homogenomic 8*x* triticale. *Genetika = Genetics (Moscow)*. 1978;14(9):1597-1603. (in Russian)

Stepochkina N.I., Stepochkin P.I. Use of microchondrometer for de-termination of grain nature of single plants of triticale and wheat. *Dostizheniya Nauki i Tekhniki APK = Achievements of Science and Technology of AIC*. 2015;29(11):39-40. (in Russian)

Torikov V.Ye., Shpilev N.S., Mameyev V.V., Yatsenkov I.N. Compara-tive characteristics of grain quality of winter triticale varieties grown in the south-west of Russia. *Vestnik Altayskogo Gosudarstvennogo Agrarnogo Universiteta = Bulletin of the Altai State Agricultural University.* 2019;2(172):49-56. (in Russian)

Trubacheeva N.V., Divashuk M.G., Chernook A.G., Pershina L.A. The effect of chromosome arm 1BS on the fertility of alloplasmic

recombinant lines in bread wheat with the *Hordeum vulgare* cytoplasm. *Plants* (*Basel*). 2021;10(6):1120. DOI 10.3390/plants10061120.

Turbayev A.Z., Sergaliev N.K., Soloviev A.A. Comparative study of some genotypes of spring triticale for grain qualities. *Izvestiya Timiryazevskoy Selskokhozyaystvennoy Akademii = Izvestiya of Timiryazev Agricultural Academy*. 2019;1:19-33. (in Russian)

Weimarck A. Elimination of wheat and rye chromosomes in a strain of octoploid *Triticale* as revealed by Giemsa banding technique. *Hereditas*. 1974;77(2):281-286. DOI 10.1111/j.1601-5223.1974.tb00940.x.

Yerzhebayeva R.S., Tajibaev D., Abugalieva A.I. Grain quality of samples of the spring triticale collection (× *Triticosecale* Wittmack)].

Sibirskii Vestnik Sel'skokhozyaistvennoi Nauki = Siberian Herald of Agricultural Science. 2020;50(3):111-121. DOI 10.26898/0370-8799-2020-3-12. (in Russian)

Zhang P., Wanlong L., Fellers J., Friebe B., Gill B.S. BAC-FISH in wheat identifies chromosome landmarks consisting of different types of transposable elements. *Chromosoma*. 2004;112(6):288-299. DOI 10.1007/s00412-004-0273-9.

Zhou J., Zhang H., Yang Z., Li G., Hu L., Lei M. Characterization of a new T2DS.2DL-?R translocation triticale ZH-1 with multiple resistances to diseases. *Genet. Resour. Crop Evol.* 2012;59:1161-1168. DOI 10.1007/s10722-011-9751-0.

Zhu F. Triticale: nutritional composition and food uses. *Food Chem.* 2018;241:468-479. DOI 10.1016/j.foodchem.2017.09.009.

ORCID ID
O.G. Silkova orcid.org/0000-0003-3299-2975
Yu.N. Ivanova orcid.org/0000-0002-9655-4539