Vavilovskii Zhurnal Genetiki i Selektsii
Vavilov Journal of Genetics and Breeding. 2024;28(4):377-386

DOI 10.18699/vjgb-24-43

Molecular cytogenetic characteristics of new spring bread wheat introgressive lines resistant to stem rust

O.A. Baranova [1], I.G. Adonina [2], S.N. Sibikeev [3]

[1] All-Russian Institute of Plant Protection, St. Petersburg-Pushkin, Russia
[2] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[3] Federal Center of Agricultural Research of the South-East Region, Saratov, Russia
baranova_oa@mail.ru

Abstract. Anticipatory wheat breeding for pathogen resistance is key to preventing economically significant crop losses caused by diseases. Recently, the harmfulness of a dangerous wheat disease, stem rust, caused by *Puccinia graminis* f. sp. *tritici*, was increased in the main grain-producing regions of the Russian Federation. At the same time, importation of the Ug99 race (TTKSK) is still a possibility. In this regard, the transfer of effective resistance genes from related species to the bread wheat breeding material followed by the chromosomal localization of the introgressions and a marker analysis to identify known resistance genes is of great importance. In this work, a comprehensive analysis of ten spring bread wheat introgressive lines of the Federal Center of Agricultural Research of the South-East Region (L657, L664, L758, L935, L960, L968, L971, L995/1, L997 and L1110) was carried out. These lines were obtained with the participation of *Triticum dicoccum*, *T. timopheevii*, *T. kiharae*, *Aegilops speltoides*, *Agropyron elongatum* and *Secale cereale*. In this study, the lines were evaluated for resistance to the Ug99 race (TTKSK) in the Njoro, Kenya. Evaluation of introgression lines in the field for resistance to the Ug99 race (TTKSK) showed that four lines were immune, two were resistant, three were moderately resistant, and one had an intermediate type of response to infection. By cytogenetic analysis of these lines using fluorescent (FISH) and genomic (GISH) *in situ* hybridization, introgressions from *Ae. speltoides* (line L664), *T. timopheevii* (lines L758, L971, L995/1, L997 and L1110), *Thinopyrum ponticum* = *Ag. elongatum* (2*n* = 70) (L664, L758, L960, L971, L997 and L1110), as well as introgressions from *T. dicoccum* (L657 and L664), *T. kiharae* (L960) and *S. cereale* (L935 and L968) were detected. Molecular markers recommended for marker-oriented breeding were used to identify known resistance genes (*Sr2*, *Sr25*, *Sr32*, *Sr1A.1R*, *Sr36*, *Sr38*, *Sr39* and *Sr47*). The *Sr36* and *Sr25* genes were observed in lines L997 and L1110, while line L664 had the *Sr39*+*Sr47*+*Sr25* gene combination. In lines L935 and L968 with 3R(3D) substitution from *S. cereale*, gene resistance was presumably identified as *SrSatu*. Thus, highly resistant to both local populations of *P. graminis* and the Ug99 race, bread wheat lines are promising donors for the production of new varieties resistant to stem rust.

Key words: *Triticum aestivum* L.; introgressive wheat lines; alien introgressions; *Puccinia graminis* f. sp. *tritici*; Ug99; *Sr* genes.

For citation: Baranova O.A., Adonina I.G., Sibikeev S.N. Molecular cytogenetic characteristics of new spring bread wheat introgressive lines resistant to stem rust. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2024;28(4):377-386. DOI 10.18699/vjgb-24-43

Funding. The work was supported by the Russian Science Foundation grant No. 22-26-00172 "Biological justification of genetic protection of wheat against stem rust in the Volga region".

Acknowledgements. Cytological analysis was performed at the Center for Microscopic Analysis of Biological Objects of the Siberian Branch of the Russian Academy of Sciences with the support of the budget project FWNR-2022-0017.
The authors thank the reviewers for their contributions to the peer review of this work.

Молекулярно-цитогенетическая характеристика новых интрогрессивных линий яровой мягкой пшеницы, устойчивых к стеблевой ржавчине

О.А. Баранова [1], И.Г. Адонина [2], С.Н. Сибикеев [3]

[1] Всероссийский научно-исследовательский институт защиты растений, Санкт-Петербург, Пушкин, Россия
[2] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[3] Федеральный аграрный научный центр Юго-Востока, Саратов, Россия
baranova_oa@mail.ru

Аннотация. Опережающая селекция пшеницы на устойчивость к патогенам – залог предотвращения экономически значимых потерь урожая от болезней. В последние годы в основных зернопроизводящих областях Российской Федерации наблюдается увеличение вредоносности опасного заболевания пшеницы – стеблевой

ржавчины (возбудитель *Puccinia graminis* f. sp. *tritici*). В то же время сохраняется опасность заноса на территорию России расы патогена Ug99 (TTKSK), которая угрожает производству зерна во всем мире. В связи с этим большое значение приобретают перенос эффективных генов резистентности от родственных видов в селекционный материал мягкой пшеницы, выявление хромосомной локализации интрогрессий и проведение маркерного анализа для идентификации известных генов устойчивости. В настоящей работе был проведен комплексный анализ десяти интрогрессивных линий яровой мягкой пшеницы селекции Федерального аграрного научного центра Юго-Востока (Л657, Л664, Л758, Л935, Л960, Л968, Л971, Л995/1, Л997 и Л1110), полученных с участием *Triticum dicoccum*, *T. timopheevii*, *T. kiharae*, *Aegilops speltoides*, *Agropyron elongatum* и *Secale cereale*. Оценка интрогрессивных линий в полевых условиях на устойчивость к расе Ug99 (TTKSK) показала, что четыре линии были иммунны, две – устойчивы, три – среднеустойчивы, а одна имела промежуточный тип реакции на заражение. Цитогенетический анализ с помощью методов флуоресцентной (FISH) и геномной (GISH) гибридизации *in situ* выявил интрогрессии от *Ae. speltoides* (линия Л664), *T. timopheevii* (линии Л758, Л971, Л995/1, Л997 и Л1110), *Thinopyrum ponticum* = *Ag. elongatum* (2*n* = 70) (Л664, Л758, Л960, Л971, Л997 и Л1110), а также интрогрессии от *T. dicoccum* (Л657 и Л664), *T. kiharae* (Л960) и *S. cereale* (Л935 и Л968). Для идентификации известных генов устойчивости (*Sr2*, *Sr25*, *Sr32*, *Sr1A.1R*, *Sr36*, *Sr38*, *Sr39* и *Sr47*) использовали молекулярные маркеры, рекомендованные для маркер-ориентированной селекции. Наличие генов *Sr36* и *Sr25* было постулировано у двух линий (Л997 и Л1110), генов *Sr39*, *Sr25* и *Sr47* – у линии Л664. У линий Л935 и Л968 с замещением 3D(3R) от *S. cereale* ген устойчивости к стеблевой ржавчине предположительно определен как *SrSatu*. Высокоустойчивые как к местным популяциям *P. graminis*, так и к расе Ug99 линии мягкой пшеницы являются перспективными донорами для создания новых устойчивых к стеблевой ржавчине сортов.

Ключевые слова: *Triticum aestivum* L.; интрогрессивные линии пшеницы; чужеродные интрогрессии; *Puccinia graminis* f. sp. *tritici*; Ug99; *Sr* гены.

Introduction

One of the conditions for increasing the yield of bread wheat is the production of varieties that are resistant to biotic and abiotic stressors. The set of the most harmful biostressors for bread wheat includes a group of rust disease pathogens: *Puccinia triticina* f. sp. *tritici* Erikss., *P. striiformis* f. sp. *tritici* Erikss., *P. graminis* f. sp. *tritici* Erikss. & Henning. These pathogens cause epiphytoties of brown, yellow and stem rust. The harmfulness of each of them can reach 50 % (Knott, 1989). The causative agents of these diseases are characterized by high virulence and great diversity in racial composition (Gultyaeva et al., 2021, 2022; Baranova et al., 2023b).

In the global production of bread wheat and under Russian conditions, a special place is occupied by stem rust (pathogen *P. graminis* f. sp. *tritici* (*Pgt*)), which can cause yield losses of more than 80 % during epiphytotic development on susceptible varieties. The well-known race of stem rust pathogen Ug99 (TTKSK) and its variants, which infect wheat varieties and lines with effective resistance genes *Sr31*, *Sr36* and *Sr24*, still pose a real threat to wheat production in the regions of the African continent, the Middle East and Asia. Due to the possibility of fungal spores spreading with air masses over vast distances, a threat of the pathogen being introduced into the territory of Eurasian countries, including Russia, remains. Over the last decade, in Europe, Kazakhstan, China and the Russian Federation, aggressive races of the fungus have appeared that are not related to the Ug99 race, but have caused severe outbreaks of the disease (Vasilova et al., 2017; Lewis et al., 2018; Baranova et al., 2021; Patpour et al., 2022).

Low diversity of stem rust resistance genes is a common problem in commercial wheat varieties around the world. The adult resistance gene *Sr57* (*Lr34/Yr18/Pm38/Bdv1*), which is part of a locus with pleiotropic action that determines nonspecific resistance to biotrophic pathogens, as well as juvenile resistance genes such as *Sr38*, *Sr6Agi*, *Sr25* and *Sr31* are used in Russian domestic varieties. The *Sr31* gene still remains effective against stem rust in the Russian Federation (Baranova et al., 2023b). Genes *Sr6Agi* and *Sr25* lose effectiveness in the Volga region, but are effective against Western Siberian populations of the fungus (Kelbin et al., 2020; Baranova et al., 2021). The *Sr38* gene is ineffective against Volga populations of the pathogen, but is recommended for breeding in Western Siberia (Skolotneva et al., 2021).

To expand the genetic basis of varieties, it is extremely important to obtain breeding material diverse for resistance genes. In general, this problem is solved by involving related species of bread wheat, mainly from the secondary and tertiary gene pools. Currently, 26 out of 63 stem rust resistance genes have been transferred from the genomes of related species (McIntosh et al., 2013, 2022). The *Ae. speltoides*, *T. timopheevii*, *T. dicoccum*, *T. ponticum*, *S. cereale* species remain important sources of valuable genes for resistance to fungal diseases and in particular to stem rust for practical breeding of bread wheat (McIntosh et al., 2013). Genes *Sr32*, *Sr39*, *Sr47* were transferred from *Aegilops speltoides* (Taush) (SS, 2*n* = 14) to the wheat genome; *Sr36*, *Sr37*, *Sr40*, from *Triticum timopheevii* Zhuk. (A^tA^t GG, 2*n* = 28); *Sr31*, *Sr27*, *Sr1A.1R*, *Sr50*, from *Secale cereale* L. (RR, 2*n* = 14) (McIntosh et al., 2013). Effectiveness against *P. graminis* and the nature and size of the introgressed material are important aspects of using these genes to develop resistant bread wheat varieties. It is important to produce combinations of currently effective *Sr* genes with each other or with genes that have partially lost their effectiveness, or with adult resistance genes.

At the Federal Center of Agricultural Research of the South-East Region (FCAR of the South-East Region), work is underway to produce new breeding material using relatives of bread wheat. Previously, lines produced with the participation of a wide range of species showed high resistance to leaf rust in the conditions of the Saratov Volga region (Gultyaeva et

Table 1. Pedigree of spring bread wheat introgressive lines

Line number	Pedigree	Source of alien genetic material
L657	L505*2//L503/3/L528//AD *T.dic*/*Ae.spelt**5S29/4/Thatcher *Lr28*	*Triticum dicoccum* Shuebl (BA), *Aegilops speltoides* Tausch (S)
L664	S55//Dobrynya/L164//Agr139/L528*2//AD *T.dic*/*Ae.spelt**5 S29//Dobrynya	*T. dicoccum* Shuebl (BA), *Ae. speltoides* Tausch (S), *Agropyron elongatum* (Host) Beauv. (source – Dobrynya variety as a carrier of translocation 7DS-7DL-7Ae#1L)
L758	L XI S29imm/L2870	*T. timopheevii*, *Ae. tauschii* (source – immune lines of Saratovskaya 29 variety (S29imm))
L935	Satu/S70//S70	*Secale cereale* L. (R) (source – triticale variety Satu)
L960	S68/*T.kiharae*//S70/3/S68	*T. kiharae* Dorof. et Migusch ((GA^{t}D)
L968	Satu/S70//S74/3/S70/4/S70	*S. cereale* L. (R), triticale variety Satu
L971	S68/*T.timopheevii**4// Dobrynya	*T. timopheevii* Zhuk. (GA^t), *Ag. elongatum* (Host) Beauv. (source – Dobrynya variety as a carrier of translocation 7DS-7DL-7Ae#1L)
L995/1	S70/Pamyati Maistrenko//S68	*T. timopheevii* and *Ae. tauschii* (source – Pamyati Maistrenko variety)
L997	S70/Pamyati Maistrenko// Dobrynya	*T. timopheevii* and *Ae. tauschii* (source – Pamyati Maistrenko variety); *Ag. elongatum* (Host) Beauv. – source of 7DS-7DL-7Ae#1L translocation, Dobrynya variety
L1110	L VI S29imm/L2032//L2032/3/L2032	*T. timopheevii*, *Ae. tauschii* (source – immune lines of Saratovskaya 29 variety (S29imm)); *Ag. elongatum* (Host) Beauv. – L2032 7DS-7DL-7Ae#1L translocation from *Ag. elongatum* (Host) Beauv.

Note. The pedigree lines indicate the following varieties of spring bread wheat: L503, L505, Dobrynya, Saratovskaya 29 (S29), Saratovskaya 55 (S55), Saratovskaya 68 (S68), Saratovskaya 70 (S70), Saratovskaya 74 (S74), as well as lines L164, L528, L2870, L2032, Agr139, L VI S29 imm, L XI S29 imm of spring bread wheat.

al., 2020). The aim of our work is a comprehensive study of new introgressive lines including assessment of resistance to the Ug99 (TTKSK) stem rust race, chromosomal localization of alien introgressions and identification of *Sr* genes using molecular markers.

Materials and methods

Plant material. Ten introgressive lines of spring bread wheat from FCAR of the South-East Region were studied. Their pedigree, indicating the donor of alien genetic material, is given in Table 1.

Cytogenetic analysis. Preparations of mitotic chromosomes were prepared from the meristem of seedling roots in accordance with the method (Badaeva et al., 2017). The FISH (fluorescence *in situ* hybridization) method using probes based on various repetitive sequences: Spelt1 (Salina et al., 1997) and Spelt52 (Salina et al., 2004), pSc119.2 (Bedbrook et al., 1980) and apAs1 (Rayburn, Gill, 1986) was used to analyze the karyotype of the lines. The FISH method described in the work of Salina et al. (Salina et al., 2006) with minor modifications was used. GISH (genomic *in situ* hybridization) using labeled *S. cereale* genomic DNA as a probe was performed according to previously published work (Schubert et al., 1998). The preparations were analyzed using an Axio Imager M1 microscope (Zeiss, Germany) equipped with a ProgRes MF CCD digital camera and Isis software (Meta Systems, Germany).

Phytopathological analysis. Resistance to race Ug99 (TTKSK) analysis was carried out at the adult plant stage using a modified Cobb scale (Peterson et al., 1948) in 2023 at the plant pathology nurseries at the International Maize and Wheat Improvement Center (CIMMYT) at the Kenya Agricultural and Livestock Research Organization (KALRO) in Njoro. The main distinguishing feature of the Ug99 race pathotypes is virulence towards carriers of the *Sr31* gene. The degree of damage to varieties with the *Sr31* gene in KALRO plant pathology nurseries in the growing season of 2023 was: for the variety Prokhorovka (*Sr31*) – 60 % (60MSS), for the variety Yugo-Vostochnaya 2 (*Sr31*) – 80 % (80S), for the variety Saratovskaya 74 (without *Sr* genes) – 80 % (80S).

Molecular genetic analysis. DNA was isolated from five-day-old wheat seedlings using cetyltrimethylammonium bromide (CTAB method) (Murray, Thompson, 1980). To identify the resistance genes *Sr2*, *Sr32*, *Sr1A.1R*, *Sr36*, *Sr38*, *Sr39*, *Sr47*, DNA markers recommended for marker-assisted selection (MAS) were used. A list of molecular markers used in the work with links to sources is presented in the Supplementary Material 1[1]. PCR was performed in duplicate on a C1000 Thermal Cycler (manufactured by BioRad). Amplification products were separated on 2 % agarose and 8 % polyacrylamide gels stained with ethidium bromide. Isogenic lines and varieties with known *Sr* genes served as a positive control; the susceptible variety Khakasskaya served as a negative control. PCR mixture was taken without adding DNA to control contamination. GeneRulerTM 50bp DNA Ladder (Thermo Scientific) was used as a molecular weight marker. Visualization of amplification products was carried out using the ChemiDoc™ (Bio-Rad) gel documentation system.

[1] Supplementary Materials 1–5 are available at: https://vavilov.elpub.ru/jour/manager/files/Suppl_Baranova_Engl_28_4.pdf

Results

Phytopathological analysis of spring bread wheat introgressive lines

Phytopathological screening of the lines at the stage of adult plants showed that all lines were resistant to the Ug99 race to varying degrees: four lines were immune (infection type 0), two were resistant (R), three were moderately resistant (MR) to this highly aggressive race of the fungus (Table 2). The only exception was one line, L995/1, which had an intermediate infection type (M) with 5 % of disease development.

Cytogenetic analysis of spring bread wheat introgressive lines

The identification of alien genetic material and determination of its state in the reconstructed genome of bread wheat in the form of addition or substitution chromosomes and translocations were the aim of the introgression lines cytogenetic analysis.

The main results of cytogenetic analysis are presented in Table 2 and in the Figure. Additional information indicating the probe combinations used is provided in the Supplementary Material 2.

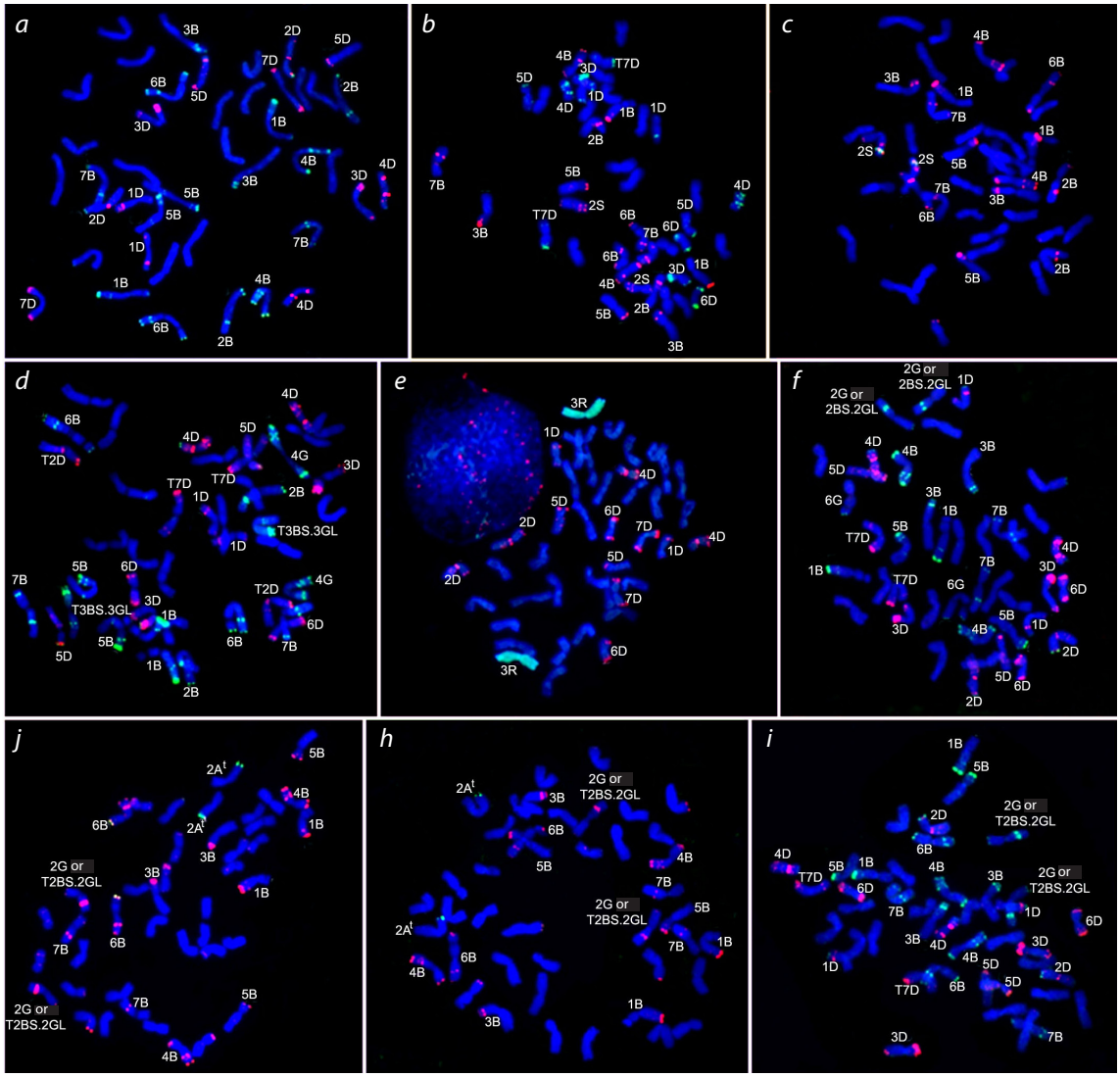
Karyotyping of the lines showed that each of them is characterized by the standard number of chromosomes for hexaploid wheat – 42. FISH was performed with probes pSc119.2 and pAs1 for each of the ten lines. The probe pSc119.2 (Bedbrook et al., 1980) is predominantly localized on the chromosomes of the bread wheat genome B, and pAs1 (Rayburn, Gill, 1986) is predominantly localized on the chromosomes of the D genome. The simultaneous use of these probes allows the identification of all chromosomes of the B and D genomes and some chromosomes of the A genome (Schneider et al., 2003). In addition, it is possible to identify the chromosomes of the G genome of *T. timopheevii* by the localization of hybridization signals with the pSc119.2 probe (Jiang, Gill, 1994). GISH with *S. cereale* DNA was used to analyze two wheat lines that had rye among ancestors. Analysis of eight lines, the ancestors of which included *Ae. speltoides*, *T. timopheevii* or *T. kiharae*, involved hybridization with probes Spelt1 and Spelt52 (performing GISH with DNA from these species is difficult due to their close relationship to bread wheat).

Table 2. Characteristics of spring bread wheat introgressive lines by translocations/substitutions, *Sr* genes and resistance to stem rust (Ug99) at the adult plants stage

Line	Cytogenetic study result	Identified *Sr* genes*	Resistance to *P. graminis* f. sp. *tritici* race Ug99 (TTKSK)**
L657	6A$^{T.\ dicoccum}$(6D)	–	5RMR
L664	2A$^{T.\ dicoccum}$(2A) or T2AS.2A$^{T.\ dicoccum}$L 2S(2D) – from *Ae. speltoides* Translocation from *Th. ponticum* to 7DL	*Sr25*, *Sr39*, *Sr47*	5RMR
L758	T2A^{t}.2A – from *T. timopheevii* Translocation from *Th. ponticum* to 7DL	*Sr25*	5R
L935	3R(3D) – from *S. cereale*	–	0
L960	2A^{t}(2A) – from *T. kiharae* T3BS.3GL – from *T. kiharae* 4G(4B) – from *T. kiharae* T2D$^{(T.\ aestivum)}$S.2D$^{(T.\ kiharae)}$L Translocation from *Th. ponticum* to 7DL	*Sr25*	5MR
L968	3R(3D) – from *S. cereale*	–	0
L971	2A^{t}(2A) – from *T. timopheevii* 2G(2B) or T2BS.2GL – from *T. timopheevii* 6G(6B) – from *T. timopheevii* Translocation from *Th. ponticum* to 7DL	*Sr25*	5R
L995/1	2A^{t}.2A – from *T. timopheevii* 2G(2B) or T2BS.2GL – from *T. timopheevii*	–	5M
L997	2A^{t}.2A – from *T. timopheevii* 2G(2B) or T2BS.2GL – from *T. timopheevii* Translocation from *Th. ponticum* to 7DL	*Sr25*, *Sr36*	0
L1110	2A^{t}.2A – from *T. timopheevii* 2G(2B) or T2BS.2GL – from *T. timopheevii* Translocation from *Th. ponticum* to 7DL	*Sr25*, *Sr36*	0

* The genes, the identification of which was confirmed by cytogenetics and pedigree analysis, are presented; the *Sr25* gene was identified previously (Baranova et al., 2023a).

** Resistance: 0 – immune infection type, R – resistant, MR – medium–resistant, RMR – intermediate type of infection between resistance and medium resistance, M – intermediate type of infection between medium resistance and medium susceptibility.



Results of FISH and GISH with different probe combinations on metaphase chromosomes of bread wheat introgressive lines.

Probes pSc119.2 (green), pAs1 (red): *a* – line L657, *b* – L664, *d* – L960, *f* – L971, *i* – L1110; probes Spelt52 (green), pSc119.2 (red): *c* – L664, *h* – L997; probes Spelt1 (green), pSc119.2 (red): *g* – L997; Rye DNA (green), pAs1 (red): *e* – line L968.

Lines L657 and L664 were obtained with the participation of *Ae. speltoides*. In the L657 line, Spelt52 repeat sites were not identified, and the Spelt1 probe is localized at the ends of the chromosome 6B arms. According to previous studies, this localization of Spelt1 occurs in bread wheat varieties (Salina et al., 2006). Consequently, we cannot speak with confidence about translocations from *Ae. speltoides* in this lineage. FISH with the pAs1 probe showed the absence of 2D chromosomes in the L664 line and revealed a pair of chromosomes with a weak signal of pSc119.2 on the short arm and two signals on the long arm (see the Figure, *b*), which also contains the Spelt52 site (see the Figure, *c*). We identified this chromosome as chromosome 2S of *Ae. speltoides* (Badaeva et al., 1996; Ruban, Badaeva, 2018). Thus, in the case of line L664, we have established chromosomal substitution 2S(2D). In addition, the results of hybridization of probes pSc119.2 and pAs1 on the chromosomes of the L657 line (see the Figure, *a*) indicate the substitution of chromosome 6D, presumably with chromosome 6A of *T. dicoccum*, a species that is present in the pedigree of this line. In the L664 line, Spelt1 sites were identified at the long arm ends of the genome A chromosomes pair, most likely chromosomes 2A. In bread wheat varieties, such localization of this probe has not been observed, but it is characteristic of tetraploid wheats, in particular *T. dicoccum*, and may indicate chromosomal substitution or translocation from a given species (present in the pedigree).

Lines L758, L960, L971, L995/1, L997, and L1110 were expected to have introgressions from the species *T. timopheevii* or *T. kiharae*. Interestingly, weak hybridization signals with the Spelt52 probe were detected on the short arms of the genome A chromosome pair, most likely chromosome 2A in all six lines (see the Figure, *h*). This localization of Spelt52 is characteristic of *T. timopheevii* or *T. kiharae* and may indicate translocations from these species. No hybridization signals with the Spelt1 probe were detected in the L758 line. Lines L995/1, L997 and L1110 carry Spelt1 blocks at the ends of the short arms of chromosome 6B (see the Figure, *g*), which is typical for a number of bread wheat varieties (Salina et al.,

2006). The localization of Spelt1 on the long arms of chromosome 2A in the lines L960, L971, L997 and L1110 (see the Figure, *g*) in combination with the localization of the Spelt52 probe on the short arms of these chromosomes (see the Figure, *h*) may indicate the substitution of chromosome 2A with $2A^t$ (from *T. timopheevii* or *T. kiharae*, respectively) in these lines. In line L960, another Spelt1 site is located on the long arm of the chromosome, which, according to the localization of the pSc119.2 probe, corresponds to chromosome 4G of *T. timopheevii*, while chromosome 4B is absent (see the Figure, *d*). The results obtained indicate that the L960 line has chromosomal substitution 4G(4B). Also, based on the localization of probe pSc119.2 in the L960 line, translocation T3BS.3GL can be assumed. The distribution of the pAs1 probe on the long arm of chromosome 2D in this line is almost identical to that in *T. kiharae*, which indicates a probable translocation $T2D^{(T.\ aestivum)}S.2D^{(T.\ kiharae)}L$ (see the Figure, *d*). It should be noted that in the L960 line, as well as in the L758, L664, L971, L997 and L1110 lines, the localization of the pAs1 probe on the long arm of chromosome 7D does not correspond to bread wheat, which indicates a translocation (see the Figure, *b*, *f*, *i*). The presence of the *Sr25* gene in four of these lines (Baranova et al., 2023a), transferred to the bread wheat genome from *Th. ponticum* (Friebe et al., 1996), and the hybridization pattern of pAs1 on the long arm of wheatgrass chromosome J^s-7 (Cui et al., 2018), allow us to conclude that chromosome 7D of these lines carries translocations from *Th. ponticum.*

In the case of the L995/1, L971 and L997, L1110 lines, according to the results of hybridization with the pSc119.2 probe, we can talk about the substitution of chromosome 2B with 2G *T. timopheevii*, 2G(2B), or about the translocation of T2BS.2GL (see the Figure, *f*–*i*). Additionally, the L971 line is expected to have a chromosomal substitution 6G(6B) (see the Figure, *f*).

Lines L935 and L968 were obtained using the Australian triticale variety Satu. GISH with rye DNA and FISH with probes pSc119.2 + pAs1 showed the substitution of 3D chromosomes with a pair of 3R chromosomes in these lines (see the Figure, *e*).

Identification of stem rust resistance genes using molecular markers

The results of *Sr* genes identification in the analyzed lines using molecular markers, confirmed by pedigree analysis and cytogenetic analysis data, are presented in Table 2. In this work, PCR fragments specific for genes *Sr32*, *Sr39*, *Sr47* (*Ae. speltoides*), *Sr36* (*T. timopheevii*) and *Sr38* (*Ae. ventricosa*) were found in different lines. All the obtained results of PCR analysis, indicating the molecular markers used, are given in the Supplementary Material 3. The diagnostic fragment of the VENTRIUP-LN2 marker for the *Sr38* gene was observed only in the L971 line (Supplementary Material 3). The presence of the *Sr36* gene was established in two lines, L997 and L1110, using the *Xstm773-2* marker (Table 2, Supplementary Material 3).

The *Sr39* gene was identified using the Sr39#22 marker. A diagnostic fragment (800 bp) was detected in five lines (Supplementary Materials 3 and 4). To identify the *Sr32* gene, the csSr32#2 marker was used. The diagnostic fragment was observed in three lines: L960, L968 and L995/1. The *Sr47* gene was identified using three markers – *Xgwm501*, *Xgpw4043*, and *Xgwm47* (Supplementary Materials 3 and 5). The diagnostic fragment of the *Xgwm501* marker (109 bp) was identified in four lines: L971, L995/1, L997 and L1110. Only the 95-bp fragment of the two diagnostic fragments of the *Xgpw4043* marker was amplified in the L657, L664, L758, and L971 lines; the 115-bp fragment was absent (Supplementary Material 5). The diagnostic fragment of the *Xgwm47* marker (165 bp) was identified only in line L664.

Previously, all the lines we analyzed were tested for the presence of the *Sr25* gene (Baranova et al., 2023a) using the Gb marker recommended for marker-based selection (Prins et al., 2001). This gene was identified in six lines (Table 2, Supplementary Material 3). According to the results of previous studies (Baranova et al., 2023a) and this work, the *Sr2*, *Sr24*, *Sr28*, *Sr31*, *Sr1A.1R* and *Sr57* genes were not found in any of the lines.

Discussion

Efficiency of molecular markers recommended for marker-based selection for identifying stem rust resistance genes

Molecular markers are widely used to identify resistance genes to various pathogens including stem rust. Among the huge number of molecular markers, the most specific ones are highlighted and recommended for marker-based selection (https://maswheat.ucdavis.edu/). However, during work with a variety of plant material, especially with introgressive lines, a researcher may encounter insufficient specificity of even a recommended marker and, as a result, false-positive gene identification. In this regard, it is desirable to conduct complex studies and confirm the presence of the desired gene along with molecular genetic analysis data, study of pedigrees, cytogenetic and phytopathological results.

During our work, introgression lines were analyzed cytogenetically and using molecular markers. Data from pedigree lines were also taken into account.

In six out of the ten studied lines (L664, L758, L960, L971, L997 and L1110), the *Sr25* gene was previously identified (Baranova et al., 2023a), which was fully confirmed by the cytogenetic analysis data in this work (Table 2). The *Sr25* gene is linked to the leaf rust resistance gene *Lr19* and is localized in the T7DS-7DL-7Ae#1L translocation from *Th. ponticum* (Friebe et al., 1994).

The identification of the *Sr36* gene using the *Xstm773-2* marker is also confirmed by cytogenetic analysis. As is known, the *Sr36* gene is localized on chromosome 2G (Friebe et al., 1996). Lines L997 and L1110, in which this gene was identified according to molecular genetic analysis, carry chromosome 2G from *T. timopheevii* (see the Figure, *g*–*i*, Table 2).

We obtained ambiguous results regarding the resistance genes *Sr32, Sr39* and *Sr47*, the source of which is *Ae. speltoides*. Cytogenetic analysis revealed genetic material from *Ae. speltoides* (substitution of chromosome 2D with chromosome 2S – 2S(2D) only in line L664, in the pedigree of which this species is present). However, the diagnostic fragment of the Sr39#22 marker (the *Sr39* gene marker) was also identified in lines L971, L995/1, L997 and L1110 (Supplementary Material 3), which lack *Ae. speltoides* in their pedigrees,

but there is genetic material from *T. timopheevii*, which was confirmed cytogenetically. Also, based on the pedigrees of the L997 and L1110 lines, Dr. Savov's synthetic (GA^{t}D) was used in crosses (*T. timopheevii* × *T. tauschii*). Thus, the diagnostic fragment of the Sr39#22 marker was amplified in lines with material from *T. timopheevii* and possibly *T. tauschii*. It should be noted that similar results for the Sr39#22 marker were obtained by E.I. Gultyaeva and colleagues (Gultyaeva et al., 2014). Their study noted that, despite the fact that this marker is widely used to identify the *Sr39/Lr35* gene, its diagnostic fragment was amplified in wheat samples with material from *T. timopheevii* and *T. tauschii*: for example, in the variety Pamyati Maistrenko, which was used to obtain lines L995/1 and L997 (Table 1).

The diagnostic fragment of the *Sr32* gene marker csSr32#2 (152 bp) was identified in lines L960, L968 and L995/1 with genetic material from *T. kiharae* and *Th. ponticum* (line L960), *S. cereale* L. (line L968) and *T. timopheevii* (line L995/1) (Supplementary Material 3). Based on all of the above, we did not take into account the results obtained for this marker of the *Sr32* gene, considering them a clear example of a false-positive gene identification.

Another gene from *Ae. speltoides* is *Sr47*, which we identified using three markers: *Xgwm501*, *Xgwm47* and *Xgpw4043*, the results were also ambiguous. The diagnostic fragment (109 bp) of the *Xgwm501* marker was clearly detected in lines L971, L995/1, L997 and L1110 (Supplementary Material 3), which were described above. The Pamyati Maistrenko variety and Dr. Savov's synthetic (GA^{t}D) – *T. timopheevii* × *T. tauschii* are present in the pedigrees of those lines. As can be seen from cytogenetic analysis, the genetic material from *Ae. speltoides* is not present in them (Table 2). As for the *Xgpw4043* marker, the diagnostic fragment of 95 bp was observed in lines L657, L664, L758 and L971, while the second diagnostic fragment of 115 bp was absent. This situation was described in the article (Klindworth et al., 2012), where in some wheat lines with the *Sr47* gene the 115-bp fragment was not amplified or differed in staining intensity and only the 95-bp fragment was amplified and the authors strongly recommended to pick up several markers for *Sr47* gene identification. The diagnostic fragment of the *Xgwm 47* marker was identified only in the L664 line, which has *Ae. speltoides* in its pedigree, and cytogenetic analysis revealed chromosomal substitution 2S(2D). Also, in this line, diagnostic fragment of the Sr39#22 marker (the *Sr39*/*Lr35* gene) was identified. It should be noted that both the *Sr47* and *Sr39* genes are localized on chromosome 2S of *Ae. speltoides*, with *Sr39* on the short arm and *Sr47* on the long arm (Klindworth et al., 2012). Since the L664 line has a 2S(2D) substitution, it is quite possible that it has both the *Sr39* and *Sr47* genes.

The *Sr38* gene comes from the species *Ae. ventricosa* Tausch. and is linked to the genes for resistance to brown (*Lr37*) and yellow (*Yr17*) rust (Bariana, McIntosh, 1993). Despite the absence of this species in the pedigrees of the introgressive lines (Table 1), the diagnostic fragment of the VENTRIUP-LN2 marker to the *Sr38* gene was present in the L971 line. Cytogenetic analysis did not reveal genetic material from *Ae. ventricosa* in this line, therefore we can conclude that in this case there was a false positive identification of the *Sr38* gene.

Thus, attention should be paid to the shown in our study insufficient specificity of the markers for the following genes: *Sr32* – csSr32#2, *Sr38* – VENTRIUP-LN2, *Sr39* – Sr39#22, *Sr47* – *Xgwm501* and *Xgpw4043*. During identification of resistance genes using molecular markers in wheat samples with alien genetic material, this point must be taken into account. It is necessary to once again note the importance of combining different approaches when conducting the analysis of introgressive forms to increase its effectiveness.

Characteristics of new spring bread wheat introgressive lines resistant to stem rust

The Supplementary Material 3 presents the results of assessing the resistance of the analyzed lines to the Volga region populations of the stem rust pathogen at the seedling stage, which we obtained earlier (Baranova et al., 2023a). It was shown that two lines (L657 and L971) were susceptible to the Tatarstan population of the fungus collected from the Nadira variety, while the L971 line was heterogeneous in resistance. Lines L758 and L960 were susceptible to the Saratov population collected from the Voevoda variety. Six lines showed resistance to both populations of the pathogen (L664, L935, L968, L995/1, L997, L1110). Thus, the characterization of introgressive lines will be based on the data from previous works (Baranova et al., 2023a, b) and the results obtained in this study.

All the lines were highly resistant to race Ug99 (TTKSK) with the exception of L995/1 as assessed by KALRO (Kenya) (Table 2). According to FAO data, to date the following genes remain effective versus the Ug99 race: *Sr28*, *Sr29*, *SrTmp* (*T. aestivum* L.), *Sr2*, *Sr13*, *Sr14* (*T. turgidum* L.), *Sr22*, *Sr35* (*T. monococcum* L.), *Sr37* (*T. timopheevii* Zhuk.), *Sr32*, *Sr39*, *Sr47*, (*Ae. speltoides* Tausch.), *Sr33*, *Sr45* (*Ae. tauschii* Coss.), *Sr40* (*T. araraticum* Jakubz.), *Sr25*, *Sr26*, *Sr43* (*Ag. elongatum* Host.), *Sr44* (*Ag. intermedium* Host.), *Sr27* and *Sr1A.1R* (*S. cereale* L.) (http://www.fao.org/agriculture/crops/rust/stem/stem-pathotypetracker/stem-effectivesrgenes/en). The *SrSatu* gene is also effective against the Ug99 race (Olivera et al., 2013).

Based on a previous analysis of the pathogen populations virulence from the Nadira and Voevoda varieties (Baranova et al., 2023b), only the *Sr32* gene is effective against both populations of the fungus among the genes, the presence of which could be assumed in the studied lines. However, this gene was not identified in any of the lines. In addition to it, the *Sr39* gene, identified only in the L664 line, is effective against the Tatarstan population of the pathogen. Consequently, the lines resistance to the pathogen is determined by other unstudied genes or combinations of genes.

In three lines resistant to the Volga region populations of the fungus (L935, L968 and L995/1), molecular genetic analysis failed to identify known resistance genes. Two of them (lines L935 and L968 (Table 1)) carry genetic material from *S. cereale*. Their pedigrees include the triticale variety Satu and they have chromosomal substitution 3R(3D) according to cytogenetic analysis (Table 2, the Figure, *e*). They also turned out to be immune to the Ug99 race. The L968 line is also immune to yellow rust (according to the KALRO assessment), i. e. it has resistance to yellow and stem rust pathogens. The *SrSatu* gene is localized on chromosome 3R of rye and is closely linked to the *LrSatu* gene. In addition, the *Sr27* gene

is localized on chromosome 3R (Singh, McIntosh, 1988). According to McIntosh (1995), the *Sr27* and *SrSatu* genes are allelic to each other and are highly effective against the stem rust pathogen populations. In earlier work, S.J. Singh and R.A. McIntosh, based on genetic analysis of F_2 and F_3 hybrids of stem rust-resistant varieties Satu (*SrSatu*) and Coorong (*Sr27*) with susceptible triticale varieties, showed that the resistance of each variety is determined by one dominant gene and the *SrSatu* and *Sr27* genes are allelic or closely linked (Singh, McIntosh, 1988). This article shows that the Satu variety used in the crosses did not carry the *Sr27* gene. In our studies, the response type was "1" to the population of *P. graminis* f. sp. *tritici* collected from the spring bread wheat variety Voevoda, and "2+" to the population from the variety Nadira for the *Sr27* gene (Baranova et al., 2023b), while lines L935 and L968 showed the infection type of either "0" or "1" (Supplementary Material 3). On the other hand, the *Sr27* gene is effective against the Ug99 race, but the lines containing it are resistant or moderately resistant (R, MR) (Jin et al., 2007); in our study, the lines were immune, the infection type was "0" (Table 2). Thus, taking into account the pedigree, as well as data from cytogenetic and phytopathological analyses, there is reason to believe that the L935 and L968 lines carry the *SrSatu* gene.

Resistance to the Volga region populations of the fungus in line L995/1 (Supplementary Material 3) is most likely determined by unidentified genes on chromosome 2G of *T. timopheevii* (chromosomal substitution 2G(2B), or translocation T2BS.2GL) (Table 2). The Pamyati Maistrenko variety is a donor of alien introgressions and resistance to the stem rust pathogen in the L995/1 line. As is known, this variety inherited chromosomal substitution 2B(2G) from the line of spring bread wheat "Saratovskaya 29 immune L10", which has age-related resistance to the stem rust pathogen (Laikova et al., 2013). The L995/1 line showed an intermediate type of resistance to the Ug99 race (5M).

Resistance genes, the presence of which is confirmed by cytogenetic analysis (Table 2) and pedigree analysis (Table 1), were identified in lines L664, L997 and L1110. Line L664 was resistant to both populations of the fungus collected from the spring bread wheat varieties Voevoda and Nadira and highly resistant (5RMR) to the Ug99 race. The following genes have been identified in this line: *Sr25* (confirmed by the presence of a translocation on 7DL from *Th. ponticum*, T7DS-7DL-7Ae#1L); *Sr39* and *Sr47*, which are located on chromosome 2S of *Ae. speltoides* (McIntosh et al., 2013). Population of *P. graminis* f. sp. *tritici* from the Voevoda variety is virulent to the *Sr39* and *Sr25* genes; however, the L664 line is highly resistant, which may be determined by an additional resistance gene from *Ae. speltoides* – *Sr47*. The type of infection for lines with the *Sr47* gene, infected with the Ug99 race, is "2–" (Klindworth et al., 2012), which correlates with the results of assessing the L664 line for resistance to Ug99 – 5RMR (Table 2). Thus, there is reason to conclude that L664 carries an effective combination of *Sr25* + *Sr39* + *Sr47* genes.

It should be noted that the use of *Sr25* gene sources in breeding for resistance to stem rust is traditional for breeding centers in the Volga region. In 2009, it was reported that a fungal isolate virulent to this gene had been identified in India (Jain et al., 2009). Unfortunately, this previously highly effective gene has been losing efficiency in recent years in the Volga region (Baranova et al., 2021; Baranova et al., 2023b). However, *Sr25* is still effective against the Ug99 race and may be valuable for breeding in combination with other genes such as *Sr31*, *Sr35* and *Sr36*, and in this case with the *Sr39* and *Sr47* genes. Lines with genetic material from *Ae. speltoides* with the *Sr39* + *Sr47* genes are very promising for breeding due to their effectiveness against the Ug99 race (Klindworth et al., 2012). Among the domestic varieties of Russia, the Chelyaba 75 variety, selected by the Chelyabinsk Research Institute of Agriculture with genetic material from *Ae. speltoides* (the *Sr39* gene), stands out and has not only group resistance to leaf and stem rusts and smut (according to the originator), but also resistance to the Ug99 race (Shamanin et al., 2011).

Lines L997 and L1110 are resistant to both Volga populations of *P. graminis* f. sp. *tritici*. Genes *Sr25* (T7DS-7DL-7Ae#1L from *Th. ponticum*) and *Sr36* (2G(2B)), or translocation T2BS.2GL were identified in those lines (Table 2, the Figure, *g–i*). Resistance to the stem rust pathogen in these lines is determined by the *Sr25* + *Sr36* genes combination and, probably, by unidentified gene(s) from *T. timopheevii* on chromosome $2A^t$. This is confirmed by the immunity of these lines to the Ug99 race, as well as the immunity of the L997 line to the yellow rust pathogen.

The resistance of the L960 line to the stem rust population from the Nadira variety and its average resistance to the Ug99 race (5MR) are most likely associated with unidentified genes from *T. kiharae* (Table 2).

Line L971 is moderately resistant to race Ug99 (Table 2). It turned out to be heterogeneous in resistance to the population of *P. graminis* f. sp. *tritici* collected from the spring bread wheat variety Nadira, and resistant to the fungal population from the Voevoda variety (Supplementary Material 3). Since it is known that both populations are virulent to the *Sr25* gene previously identified in this line, it can be assumed that it has other resistance genes, most likely localized on chromosome $2A^t$ of *T. timopheevii* and/or on chromosome 2G (Table 2). Moreover, the resistance gene(s) differ from the genes previously transferred from *T. timopheevii*, *Sr36* (T2B/2G#1) and *Sr40* (T2BL/2G#2S), by greater efficiency, since populations of *P. graminis* f. sp. *tritici* collected from varieties Nadira and Voevoda are virulent to them (Baranova et al., 2023b).

The resistance of the L758 line to the Ug99 race (5R) is determined by the *Sr25* gene – T7DS-7DL-7Ae#1L from *Th. ponticum* (Table 2).

Line L657 is resistant to the stem rust population collected from the Voevoda wheat variety and to the Ug99 race (5RMR), but does not carry any of the tested *Sr* genes. It is possible that unidentified or unknown *Sr* genes that determine the resistance of this line are localized on chromosome 6A of *T. dicoccum*, which replaced its chromosome 6D (Table 2, the Figure, *a*).

Thus, as a result of phytopathological, molecular genetic and cytogenetic analyses, lines that were immune and resistant to the Volga region populations of the fungus, as well as to the Ug99 race (Table 2), with effective combinations of resistance

genes: *Sr25* + *Sr39* + *Sr47* (L664), *Sr25* + *Sr36* (L997 and L1110) and with a gene, previously identified as *SrSatu* (L935 and L968), were isolated. Lines L657, L960, and L971 may be sources of new stem rust resistance genes.

Conclusion

Cytogenetic analysis together with identification using DNA markers of *Sr* genes and phytopathological evaluation of resistance to the Ug99 *P. graminis* f. sp. *tritici* race in introgressive lines of spring bread wheat made it possible to: determine the nature of alien introgressions; establish the degree of resistance to the pathogen; identify effective *Sr* genes. As a result, comprehensive characterization of ten introgressive lines of spring bread wheat resistant to the Ug99 race was obtained, which allows their targeted use in the breeding of spring bread wheat for resistance to the stem rust pathogen.

References

Badaeva E.D., Friebe B., Gill B.S. Genome differentiation in *Aegilops*. 1. Distribution of highly repetitive DNA sequences on chromosome of diploid species. *Genome*. 1996;39(2):293-306. DOI 10.1139/g96-040

Badaeva E.D., Ruban A.S., Aliyeva-Schnorr L., Municio C., Hesse S., Houben A. In situ hybridization to plant chromosomes. In: Liehr T. (Ed.) Fluorescence In Situ Hybridization (FISH): Application Guide. Springer protocols handbooks. Berlin; Heidelberg: Springer-Verlag, 2017;477-494. DOI 10.1007/978-3-662-52959-1_49

Baranova O.A., Sibikeev S.N., Druzhin A.E., Sozina I.D. Loss of effectiveness of stem rust resistance genes *Sr25* and *Sr6Agi* in the Lower Volga region. *Vestnik Zashchity Rasteniy = Plant Protection News.* 2021;104(2):105-112. DOI 10.31993/2308-6459-2021-104-2-14994 (in Russian)

Baranova O.A., Sibikeev S.N., Konkova E.A. Analysis of resistance to stem rust and identification of *Sr* genes in introgressive lines of spring bread wheat. *Trudy po Prikladnoy Botanike, Genetike i Selektsii = Proceedings on Applied Botany, Genetics and Breeding*. 2023a;184(1):177-186. DOI 10.30901/2227-8834-2023-1-177-186 (in Russian)

Baranova O., Solyanikova V., Kyrova E., Kon'kova E., Gaponov S., Sergeev V., Shevchenko S., Mal'chikov P., Dolzhenko D., Bespalova L., Ablova I., Tarhov A., Vasilova N., Askhadullin Dm., Askhadullin Dn., Sibikeev S. Evaluation of resistance to stem rust and identification of *Sr* genes in Russian spring and winter wheat cultivars in the Volga region. *Agriculture*. 2023b;13(3):635. DOI 10.3390/agriculture13030635

Bariana H.S., McIntosh R.A. Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. *Genome*. 1993;36(3):476-482. DOI 10.1139/g93-065

Bedbrook R.J., Jones J., O'Dell M., Thompson R.J., Flavell R.B. A molecular description of telomeric heterochromatin in *Secale* species. *Cell*. 1980;19(2):545-560. DOI 10.1016/0092-8674(80)90529-2

Cui Y., Zhang Y., Qi J., Wang H., Wang R.R.C., Bao Y., Li X. Identification of chromosomes in *Thinopyrum intermedium* and wheat *Th. intermedium* amphiploids based on multiplex oligonucleotide probes. *Genome.* 2018;61(7):515-521. DOI 10.1139/gen-2018-0019

Friebe B., Jiang J., Knott D.R., Gill B.S. Compensation indexes of radiation-induced wheat *Agropyron elongatum* translocations conferring resistance to leaf rust and stem rust. *Crop Sci.* 1994;34(2):400-404. DOI 10.2135/cropsci1994.0011183X003400020018x

Friebe B., Jiang J., Raupp W.J., McIntosh R.A., Gill B.S. Characterization of wheat-alien translocations conferring resistance to diseases and pests current status. *Euphytica*. 1996;91:59-87. DOI 10.1007/BF00035277

Gultyaeva E.I., Orina A.S., Gannibal Ph.B., Mitrofanova O.P., Odintsova I.G., Laikova L.I. The effectiveness of molecular markers for the identification of *Lr28*, *Lr35*, and *Lr47* genes in common wheat. *Russ. J. Genet*. 2014;50(2):131-139. DOI 10.1134/S1022795414020069

Gultyaeva E.I., Sibikeev S.N., Druzhin A.E., Shaydayuk E.L. Enlargement of genetic diversity of spring bread wheat resistance to leaf rust (*Puccinia triticina* Eriks.) in Lower Volga region. *Selskokhozyaystvennaya Biologiya = Agricultural Biology.* 2020;55(1):27-44. DOI 10.15389/agrobiology.2020.1.27eng

Gultyaeva E.I., Shaydayuk E.L., Gannibal P.B. Leaf rust resistance genes in wheat cultivars registered in Russia and their influence on adaptation processes in pathogen populations. *Agriculture*. 2021;11(4):319. DOI 10.3390/agriculture11040319

Gultyaeva E., Shaydayuk E., Kosman E. Virulence diversity of *Puccinia striiformis* f. sp. *tritici* in common wheat in Russian regions in 2019–2021. *Agriculture*. 2022;12(11):1957. DOI 10.3390/agriculture12111957

Jain S.K., Prashar M., Bhardwaj S.C., Singh S.B., Sharma Y.P. Emergence of virulence to *Sr25* of *Puccinia graminis* f. sp. *tritici* on wheat in India. *Plant Dis*. 2009;93(8):840. DOI 10.1094/PDIS-93-8-0840B

Jiang J., Gill B.S. Different species-specific chromosome translocation in *Triticum timopheevii* and *T. turgidum* support diphyletic origin of polyploid wheats. *Chromosome Res*. 1994;2(1):59-64. DOI 10.1007/BF01539455

Jin Y., Singh R.P., Ward R.W., Wanyera R., Kinyua M., Njau P., Fetch T., Pretorius Z.A., Yahyaoui A. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Dis.* 2007;91(9):1096-1099. DOI 10.1094/PDIS-91-9-1096

Kelbin V.N., Skolotneva E.S., Salina E.A. Challenges and prospects for developing genetic resistance in common wheat against stem rust in Western Siberia. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2020;24(8):821-828. DOI 10.18699/VJ20.679 (in Russian)

Klindworth D.L., Niu Z., Chao S., Friesen T.L., Faris J.D., Cai X., Xu S.S. Introgression and characterization of a goatgrass gene for a high level of resistance to Ug99 stem rust in tetraploid wheat. *G3* (*Bethesda*). 2012;2(6):665-673. DOI 10.1534/g3.112.002386

Knott D.R. The Wheat Rust – Breeding for Resistance. (Ser. Monographs on Theoretical and Applied Genetics). Berlin: Springer-Verlag, 1989. DOI 10.1007/978-3-642-83641-1

Laikova L.I., Belan I.A., Badaeva E.D., Rosseeva L.P., Shepelev S.S., Shumny V.K., Pershina L.A. Development and study of spring bread wheat variety Pamyati Maystrenko with introgression of genetic material from synthetic hexaploid *Triticum timopheevii* Zhuk. × *Aegilops tauschii* Coss. *Russ. J. Genet.* 2013;49(1):89-97. DOI 10.1134/S1022795413010067

Lewis C.M., Persoons A., Bebber D.P., Kigathi R.N., Maintz J., Findlay K., Bueno-Sancho V., Corredor-Moreno P., Harrington S.A., Kangara N., Berlin A., García R., German S.E., Hanzalová A., Hodson D.P., Hovmøller M.S., Huerta-Espino J., Imtiaz M., Mirza J.I., Justesen A.F., Niks R.E., Omarani A., Patpour M., Pretorius Z.A., Roohparvar R., Sela H., Singh R.P., Steffenson B., Visser B., Fenwick P.M., Thomas J., Wulff B.B.H., Saunders D.G.O. Potential for re-emergence of wheat stem rust in the United Kingdom. *Commun. Biol*. 2018;1:13. DOI 10.1038/s42003-018-0013-y

McIntosh R.A., Wellings C.R., Park R.F. Wheat Rusts: An atlas of resistant genes. CSIRO Publication, Collingwood, 1995. DOI 10.1007/978-94-011-0083-0

McIntosh R.A., Yamazaki Y., Dubcovsky J., Rogers W.J., Morris C., Appels R., Xia X.C. Catalogue of Gene Symbols for Wheat. 12th International Wheat Genetics Symposium. 8–13 September 2013. Yokohama, Japan, 2013. https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/2013/GeneCatalogueIntroduction.pdf

McIntosh R.A., Dubcovsky J., Rogers W.J., Xia X.C., Raupp W.J. Catalogue of Gene Symbols for Wheat: 2022 Supplement. *Annu. Wheat Newsl*. 2022;68:68-81

Murray M.G., Thompson W.F. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*. 1980;8(19):4321-4325. DOI 10.1093/nar/8.19.4321

Olivera P.D., Pretorius Z.A., Badebo A., Jin Y. Identification of resistance to races of *Puccinia graminis* f. sp. *tritici* with broad virulence in triticale (×*Triticosecale*). *Plant Dis*. 2013;97:479-484. DOI 10.1094/PDIS-05-12-0459-RE

Patpour M., Hovmøller M.S., Rodriguez-Algaba J., Randazzo B., Villegas D., Shamanin V.P., Berlin A., Flath K., Czembor P., Hanzalova A., Sliková S., Skolotneva E.S., Jin Y., Szabo L., Meyer K.J.G., Valade R., Thach T., Hansen J.G., Justesen A.F. Wheat stem rust back in Europe: diversity, prevalence and impact on host resistance. *Front. Plant Sci.* 2022;13:882440. DOI 10.3389/fpls.2022.882440

Peterson R.F., Campbell A.B., Hannah A.E. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res*. 1948;26(5):496-500. DOI 10.1139/cjr48c-033

Prins R., Groenewald J.Z., Marais G.F., Snape J.W., Koebner R.M.D. AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. *Theor. Appl. Genet.* 2001;103(4):618-624. DOI 10.1007/PL00002918

Rayburn A.L., Gill B.S. Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. *Plant Mol. Biol. Rep*. 1986;4(2):104-109. DOI 10.1007/BF02732107

Ruban A.S., Badaeva E.D. Evolution of the S-genomes in *Triticum-Aegilops* alliance: evidences from chromosome analysis. *Front. Plant Sci*. 2018;9:1756. DOI 10.3389/fpls.2018.01756

Salina E.A., Pestsova E.G., Vershinin A.V. "Spelt1" a novel family of tandemly repeated DNA in cereals. *Russ. J. Genet*. 1997;33(4):352-357

Salina E.A., Adonina I.G., Vatolina T.Y., Kurata N. Comparative analysis of the composition and organization of two subtelomeric repeat families in *Aegilops speltoides* Tausch and related species. *Genetica*. 2004;122(3):227-237. DOI 10.1007/s10709-004-5602-7

Salina E.A., Lim Y.K., Badaeva E.D., Scherban A.B., Adonina I.G., Amosova A.V., Samatadze T.E., Vatolina T.Y., Zoshchuk S.A., Leitch A. Phylogenetic reconstruction of *Aegilops* section *Sitopsis* and the evolution of tandem repeats in the diploids and derived wheat polyploids. *Genome*. 2006;49(8):1023-1035. DOI 10.1139/g06-050

Schneider A., Linc G., Molnar-Lang M. Fluorescence *in situ* hybridization polymorphism using two repetitive DNA clones in different cultivars of wheat. *Plant Breed.* 2003;122(5):396-400. DOI 10.1046/j.1439-0523.2003.00891.x

Schubert I., Shi F., Fuchs J., Endo T.R. An efficient screening for terminal deletions and translocations of barley chromosomes added to common wheat. *Plant J*. 1998;14(4):489-495. DOI 10.1046/j.1365-313X.1998.00125.x

Shamanin V.P., Morgunov A.I., Chursin A.S., Merkeshina N.N., Shtubey T.Yu., Levshunov M.A., Karakoz I.I. Does stem rust pose a threat to the wheat harvest in Western Siberia. *Uspekhi Sovremennogo Estestvoznaniya = Advances in Current Natural Sciences*. 2011;2:56-60 (in Russian)

Singh S.J., Mclntosh R.A. Allelism of two genes for stem rust resistance in triticale. *Euphytica*. 1988;38:185-189. DOI 10.1007/BF00040190

Skolotneva E.S., Kelbin V.N., Shamanin V.P., Boyko N.I., Aparina V.A., Salina E.A. The gene *Sr38* for bread wheat breeding in Western Siberia. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2021;25(7):740-745. DOI 10.18699/VJ21.084

Vasilova N.Z., Askhadullin Dam.F., Askhadullin Dan.F. Stem rust epiphytotic on soft spring wheat in Tatarstan. *Zashchita i Karantin Rasteniy = Plant Protection and Quarantine*. 2017;2:27-28 (in Russian)

Conflict of interest. The authors declare no conflict of interest.

Received November 7, 2023. Revised March 5, 2024. Accepted March 5, 2024.