RUDN Journal of Agronomy and Animal Industries. ISSN 2312-797X (Print). ISSN 2312-7988 (Online)
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2025;20(4):577–588
http://agrojournal.rudn.ru

Genetics and plant breeding
Генетика и селекция растений

DOI: 10.22363/2312-797X-2025-20-4-577-588
EDN CABXNN
UDC 633.11./631.527

Research article / Научная статья

Identification of genes for resistance to leaf and stem rust in breeding lines of spring common wheat from the secondary gene pool of Arsenal collection

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Abstract. Leaf rust (*Puccinia triticina* Eriks.) and stem rust (*Puccinia graminis* f. sp. *tritici*) are the main causes of declining wheat yields in Russia and abroad. Epidemics of these diseases lead to significant economic losses. In recent years, there has been increased pressure from new, more aggressive races of pathogens. As a result, a breeding strategy aimed at protecting wheat varieties from these diseases is extremely relevant and a priority. The aim of this work was to identify effective and partially effective genes of resistance to leaf (*Lr19*, *Lr24*, *Lr26*, *Lr29*, *Lr34*, *Lr37*) and stem (*Sr17*, *Sr22*, *Sr36*, *Sr39*, *Sr47*) rust in the Ob forest-steppe of the Altai Territory for the further development of a set of breeding measures aimed at creating new varieties of spring wheat with resistance to rust diseases in local conditions using modern methods of molecular marker selection. The material for the study was 25 promising lines of spring common wheat of secondary origin from Arsenal collection (Nemchinovka Research Center) with group resistance to leaf-stem diseases and having genetic material of the species *Aegilops speltoides*, *Ae. triuncialis*, *Triticum kiharae*, *Secale cereale* and *T. migushovae* in the pedigree. Molecular analysis made it possible to determine effective *Lr* genes in 80 %, and *Sr* genes in all tested accessions. The largest number of identified genes (5–6) were found in the following lines: 5–16i,

20–16i, 34–16i, 44–16i, 45–16i, 48–16i, 53–16i, and the smallest (2–3) in: 1–16i, 14–16i, 19–16i, 21–16i, 25–16i, 40–16i, 49–16i, 61–16i and 135/10i. The desired *Lr* genes were not found in accessions 1–16i, 28–16i, 49–16i, 61–16i. The results of the studies showed the presence of a wide range of genes of resistance to leaf and stem rust, which indicates the donor properties of the lines of Arsenal collection and the possibility of their effective use in marker associated selection in the development of wheat varieties resistant to rust diseases.

Keywords: DNA-markers, plant diseases, agricultural genetics, fungal pathogens, *Lr*-genes, *Sr*-genes, marker-assisted selection

Authors' contribution: Petin V.A. — conducting experiments, scientific writing; Lepekhov S.B. — concept development, verification and editing of the manuscript; Lapochkina I.F., Gainullin N.R. — discussion and approval of the final version of the manuscript.

Funding. The research was supported by the Russian Ministry of Education and Science's project "Development of a Breeding and Seed Center for Grain and Legume Crops — a division of Altai Research Institute of Agriculture". (Agreement No. 075-15-2025-186 dated April 18, 2025)

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

Article history: received 19 June 2025; accepted 15 October 2025.

For citation: Petin VA, Lepekhov SB, Lapochkina IF, Gainullin NR. Identification of genes for resistance to leaf and stem rust in breeding lines of spring common wheat from the secondary gene pool of Arsenal collection. *RUDN Journal of Agronomy and Animal Industries*. 2025;20(4):577–588. doi: 10.22363/2312-797X-2025-20-4-577-588 EDN: CABXNN

Introduction

Leaf rust (*Puccinia triticina* Eriks.) and stem rust (*Puccinia graminis* f. sp. *tritici*) are regularly occurring and dangerous wheat diseases both in Russia and abroad. As part of programs to ensure the overall quality of wheat, advanced breeding lines and various wheat varieties are being tested for resistance to common rust species.

To slow the growth of pathogens that affect grain crops and prevent the emergence of new, more dangerous races, it is necessary to employ strategies aimed at increasing the genetic stability of the agrocenosis. These include a partial mixture of varieties and the cultivation of varieties with different levels of resistance to survival within a single area (variety mosaic strategy). In advanced countries, the differences are 3–4 years, while in Russia this period is significantly longer — 7–10 years or more [1]. Breeding efforts aimed at developing spring wheat varieties demonstrate a diverse genetic basis for resistance to leaf and stem diseases, which is highly relevant.

More than 80 genes of resistance to leaf and stem rust with established localization on wheat chromosomes are known [2]. West Siberian populations of leaf and stem rust are distinguished by particular virulence and can overcome plant resistance genes that are effective in other regions. Genes of juvenile resistance (*Lr24, Lr28, Lr41, Lr45, Lr47, LrAg, LrAeg.speltoides*) and age-related resistance (*Lr35, Lr48, Lr49*) have been identified that are highly effective against *Puccinia triticina* [3]. In conditions of the

southern forest-steppe of Western Siberia, spring wheat lines with the following genes showed immunity to stem rust: *Sr9e, Sr24, Sr25, Sr26, Sr27, Sr31, Sr33, Sr35, Sr36, Sr38, SRDP-2* with the *Sr7a+Sr12+Sr* gene pyramid [4]. According to the results of our own evaluations of the collection of near-isogenic lines with the *Lr* and *Sr* genes in 2022, the following genes showed high efficiency: *Lr9, Lr19, Lr24, Lr25, Lr26, Lr28, Lr29, Lr35, Lr37, Lr44, Lr45, Lr47* and *Sr24, Sr31, Sr36*.

The aim of this study was to identify several known effective and partially effective genes for resistance to leaf (*Lr19, Lr24, Lr26, Lr29, Lr34, Lr37*) and stem (*Sr17, Sr22, Sr36, Sr39, Sr47*) rust in spring common wheat lines from the secondary gene pool of the Arsenal collection using modern diagnostic methods. The obtained results will serve as the basis for developing a set of measures aimed at creating new spring wheat varieties with resistance to leaf and stem rust in the conditions of the Ob forest-steppe of Altai Krai using the MAS method.

Materials and methods

The research was conducted in 2024–2025 at the molecular genetics laboratory of Altai Research Institute of Agriculture. To identify the *Lr* and *Sr* genes, 25 spring common wheat lines from the secondary gene pool of Arsenal collection, developed at Nemchinovka Research Center through multi-stage hybridization of donors of resistance to the Ug99 race of stem rust, were used. These breeding lines possess group resistance to European and West Siberian populations of leaf and stem rust [5], which was confirmed by field studies in the experimental field of Altai Research Institute of Agriculture in 2021–2022. The CIMMYT scale [6] was used to assess the response and degree of damage by rust species.

Wheat DNA was isolated from 6–7-day-old etiolated seedlings using the method of Plaschke et al. [7]. Gene identification was performed using the polymerase chain reaction (PCR) method with primers marking the *Lr* genes: *Lr19, Lr24, Lr26, Lr29, Lr34, Lr37* and the *Sr* genes: *Sr17, Sr22, Sr36, Sr39, Sr47*. Primers were selected based on literature data; their nucleotide sequences are shown in Table 1.

Table 1

PCR-markers used to identify *Lr* and *Sr*-genes

Gene	Markers			Source
	Marker ID	Primer sequences (5'-3')	Product size, bp	
*Lr*19	SCS265-FSCS265-R	GGCGGATAAGCAGAGCAGAGGGCGGATAAGTG GGTTATGG	512	[8]
*Lr*24	SCS73-FSCS73-R	TCGTCCAGATCAGAATGTGCTCGTCGATTAGCA GTGAG	719	[9]
*Lr*26	SCM9-FSCM9-R	TGACAACCCCCTTTCCCTCGTTCATCGACGCTAA GGAGGACCC	207	[10]
*Lr*29	Lr29F24Lr29R24	GTGACCTCAGGCAATGCACACAGTGTGACCTCA GAACCGATGTCCATC	900	[11]

Ending tabl. 1

Gene	Markers			Source
	Marker ID	Primer sequences (5'-3')	Product size, bp	
*Lr*34	csLV34-FcsLV34-R	GTTGGTTAAGACTGGTGATGGTGCTTGCTATTGCTGAATAGT	150	[12]
*Lr*37	VentriupLN2	AGGGGCTACTGACCAAGGCTTGCAGCTACAGCAGTATGTACACAAAA	259	[13]
*Sr*17	WPT5343-FWPT5343-R	TATTCTACAACGCTCCATCCCGCATGCAANCCATACCTTT	407	[14]
*Sr*22	WMC633-FWMC633-R	ACACCAGCGGGGATATTTGTTACGTGCACAAGACATGAGGTGGATT	117	[15]
*Sr*36	XSTM773–2FXSTM773–2R	ATGGTTTGTTGTGTTGTGTGTAGGAAACGCCCCAACCACCTCTCTC	155	[16]
*Sr*39	SR39#22-FSR39#22-R	AGAGAAGATAAGCAGTAAACATGTGCTGTCATGAGAGGAACTCTG	487	[17]
	Sr39#50s-FSr39#50s-R	CCAATGAGGAGATCAAAACAACCCTAGCAAGGACCAAGCAATCTTG	167	
*Sr*47	WGWM501-FWGWM501-R	GGCTATCTCTGGCGCTAAAATCCACAAACAAGTAGCGCC	109	[18]

Source: compiled by V.A. Petin.

Results and Discussion

Based on our evaluation of the breeding lines, the entire collection demonstrated high resistance to leaf and stem rust in the Ob forest-steppe conditions of the Altai Krai in 2021 and 2022, compared to the standard variety, Altayskaya 70, which was 60–70% susceptible to leaf rust and 40% susceptible to stem rust (Table 2). Based on this, it was concluded that these lines possess effective resistance genes, *Lr* and *Sr*, or combinations thereof.

Table 2

Results of the evaluation of resistance of lines from Arsenal collection to leaf and stem rust, experimental field of Altai Research Institute of Agriculture, 2021–2022

Variety/Line	Pedigree*	Evaluation of resistance to leaf rust, %/type		Evaluation of resistance to stem rust, %/type	
		2021	2022	2021	2022
Altaiskaya-70 (susceptible standart)	Altaiskaya-98/ Altaiskaya-325	70S	60S	40S	40S
1–16i	(96/113)/145//113	10MR	5MR	5MR	5MR
5–16i	(96/113)/145	5MR	R	5MR	5MR
6–16i	(96/113)/145	R	R	0	0
14–16i	96/113	R	R	R	R

Ending tabl. 2

Variety/Line	Pedigree*	Evaluation of resistance to leaf rust, %/type		Evaluation of resistance to stem rust, %/type	
		2021	2022	2021	2022
17–16i	96/113	R	R	0	0
19–16i	96/113	0	0	R	R
20–16i	96/113	R	R	R	0
21–16i	96/113	0	R	0	R
25–16i	96/113	0	R	R	R
28–16i	(113/96)/145//113	R	R	R	0
30–16i	(113/96)/113	R	R	0	0
31–16i	(113/96)/113	R	R	R	R
34–16i	(113/96)/113	5MR	5MR	0	0
36–16i	(113/96)/145	R	R	R	R
37–16i	(113/96)/145	R	5MR	R	0
40–16i	(113/96)/145	R	R	5MR	5MR
44–16i	(113/119)/113	0	0	0	0
45–16i	(113/119)/113	0	0	R	0
48–16i	(113/119)/113	0	R	0	0
49–16i	(113/119)/113	R	5MR	5MR	5MR
53–16i	(113/119)/113	5MR	R	5MR	5MR
57–16i	(113/119)/119	R	R	0	0
60–16i	(119/113)/113/113	0	R	5MR	5MR
61–16i	(119/96)/113	R	R	0	0
135/10i	102/00i /Estivum 440	R	R	0	0

Note. *The following donors of resistance to leaf and stem rust participated in the creation of the lines: GT 96/90 winter wheat line (Bulgaria) with ***T. migushovae*** genetic material (in the table it is listed as line 96); Line 113/00i-4 is a sample of spring wheat from the Arsenal collection with genetic material of the species ***Ae. triuncialis*** and ***T. kiharae*** (in the table — line 113); The spring wheat line 145/05i is the result of crossing the Lada spring variety with a sample from the Arsenal collection k-62903, which was obtained with the participation of the ***Ae. speltoides*** species (in the table — line 145); The winter wheat line 119/4–06rw is a three-genera wheat-aegilops-rye hybrid containing foreign material *Ae. speltoides* and ***S. cereale*** (in the table — line 119). The spring wheat line 135/10i is the result of crossing a sample from the Arsenal collection 102/00i (with genetic material ***Ae. speltoides***) with Estivum 440 spring wheat [Chaika(w)/Tselinnaya 20/3/ Yubileynaya Osetii (w)//Bezostaya 1/Saratovskaya 36].

Source: compiled by V.A. Petin.

In wheat breeding, the genetic diversity of wild and cultivated relatives, as well as other cereal crops, is widely used to improve resistance to pathogens. The 1RS/1BL translocation, carrying the *Lr26*, *Sr31*, *Yr9*, and *Pm8* genes, which provide resistance to powdery mildew and rust diseases, is one of the most frequently used genetic elements in breeding programs worldwide [19]. Although the Sec-1 locus, encoding ε-secalin

(secalins are storage proteins in rye grain), has a negative effect on the baking qualities of wheat, the 1RS/1BL translocation generally has a positive effect on yield and adaptability to environmental conditions [20].

Identification of the *Lr26* gene was carried out using the SCM9 marker, which allows differentiation of genotypes carrying 1BL.1RS and 1AL.1RS translocations. An amplicon of 207 bp indicates the presence of the 1BL.1RS translocation, and 228 bp indicates the 1AL.1RS translocation [21]. As a result of PCR, an amplification fragment of only 207 bp in size was detected in 16 lines: 5-16i, 53-16i, 6-16i, 14-16i, 17-16i, 19-16i, 20-16i, 21-16i, 25-16i, 30-16i, 31-16i, 36-16i, 37-16i, 44-16i, 45-16i, 48-16i. Common wheat received the translocation 1RS from the rye variety Petkus, which is located in the long arm of chromosome 1B. This translocation also contains genes for resistance to powdery mildew *Pm8*, stem (*Sr31*) and yellow (*Yr9*) rust [2]. Genes for resistance to three types of rust are independent but closely linked to each other. The 1BL.1RS translocation, among other things, contains genes that favorably influence yield, grain quality, and drought tolerance, which is achieved through increased root mass [20].

The *Lr34* gene confers slow-rusting resistance to wheat plants, which is characterized by an extended period of disease development after infection, as well as a reduction in the number and size of pathogen pustules on the leaf surface. Using this gene in combination with other leaf rust resistance genes (*Lr10*, *Lr13*, *Lr16*, etc.) will allow the development of less susceptible wheat samples and varieties [22].

The *Lr34* leaf rust resistance gene is considered to be of low efficacy in Russia [23], but it is valuable as a source of other disease resistance genes linked to it in the same chromosome segment, such as yellow rust (*Yr18*), powdery mildew (*Pm46*), and stem rust (*Sr57*) [5]. Identification of the *Lr34* gene was performed using the codominant STS marker csLV34, which identifies the gene in various allelic states. The dominant (functional) allele is indicated by the presence of amplification fragment with a molecular weight of 150 bp in the samples, while the recessive (non-functional) allele is indicated by 229 bp fragment. The detection of both allelic variants of the *Lr34* gene in a sample may indicate heterozygosity at this locus, or it may be a consequence of heterogeneity in the source material due to DNA extraction from several seedlings with different genotypes.

Almost all of the presented lines possessed the recessive allele of the Lr34 gene. The dominant allele was detected only in lines 34–16i, 40–16i, and 135/10, while the heterozygous allelic state was observed only in the control sample.

The Lr37 gene was transferred to common wheat via the 2NS-2AS translocation from *Aegilops ventricosa* as part of the *Yr17/Lr37/Sr38* gene cluster and is localized on the short arm of chromosome 2A. Until recently, it was considered highly effective in many countries. However, the widespread cultivation of varieties with *Lr37* gene in Western Europe has led to a loss of its effectiveness. In Russia, the effectiveness of *Lr37* gene varies by region from high to moderate [24].

Identification of the *Lr37* gene in collection lines was performed using the VENTRIUP and LN2 primers. A 259 bp marker fragment was detected in 11 genotypes: 5-16i, 34-16i, 53-16i, 60-16i, 17-16i, 30-16i, 31-16i, 44-16i, 45-16i, 48-16i, and 57-16i.

The *Lr19, Lr24*, and *Lr29* genes were introgressed into wheat from *Agropyrum elongatum*. All of them are located in the D genome and are closely linked to the stem rust resistance genes *Lr19/Sr25* and *Lr24/Sr24*. These genes are considered highly effective and are widely used in breeding. To identify these genes, the SCAR markers SCS265 (*Lr19*), SCS73 (*Lr24*), and Lr29F24 (*Lr29*) were used. Amplification fragments for all three genes were detected only in control samples, indicating the absence of *Agropyrum elongatum* genetic material in the pedigrees of the studied lines.

The *Sr17* gene (from *T. turgidum*), located on chromosome 7B and linked to *Lr14a*/*Pm5*, although ineffective against Ug99, can provide resistance to local populations in certain regions and in combination with other genes, such as *Sr13* [25]. The *Sr17* gene was detected using the DArT marker wPt 5343 in four lines: 20-16i, 34-16i, 44-16i, and 53-16i.

The *Sr22* gene was originally identified in *Triticum monococcum* and was then transferred to tetraploid and hexaploid wheat through interspecific hybridization. This gene is effective against all races of stem rust, and there are lines with *Sr22* that are not linked to undesirable agronomic traits [26].

Sr22 was previously mapped to the long arm of chromosome 7A. Three linked markers, CFA2019, CFA2123, and BARC121, were used to haplotype this locus [27]. Olson et al. [15] generated a new set of lines with reduced foreign fragments and found that the closest markers adjacent to *Sr22* in these lines were WMC633 and CFA2123.

When using marker CFA2123, a characteristic 234 bp product was found. We observed this in all the samples studied, including the negative control. Since this marker is not fully diagnostic and can give false-positive results, we used another SSR marker, WMC633. As a result, we detected amplified products that were described as not only diagnostic. All lines had fragments of approximately 240 bp, and 117 bp were absent only in 21–16i, 40–16i, 34–16i, and 17–16i. As Olson et al. [15] wrote, this may be due to recombination between the resistance gene and all markers mapped in this region.

Sr36 was introgressed into wheat from *T. timopheevii* and is localized on the short arm of chromosome 2B. This gene is widely used in breeding and is distributed in many varieties worldwide. *Sr36* is effective against most stem rust races, including Ug99, except for the TTKST and TTTSK varieties [28]. It is also used to create pyramids in combination with other *Sr* genes during the breeding of resistant wheat varieties.

To detect this gene, the SSR codominant marker Xstm 773–2 was used, yielding clear, readable fragments of 155 bp in length, indicating the presence of the gene, and 190 bp in length, indicating the absence of the gene. The 155 bp fragment was amplified in most accessions, while the 190 bp fragment was detected in 25–16i and 135/10i.

The stem rust resistance gene *Sr39* confers resistance to all known pathotypes of *Puccinia graminis* f. sp. *tritici*, including Ug99 (TTKSK) and its variants, TTKST and TTTSK, which are virulent against *Sr24* and *Sr36*, two frequently used resistance genes. The *Sr39* gene was transferred into the hexaploid wheat cultivar Marquis to chromosome 2B from the 2S chromosome of *Aegilops speltoides* [29]. The transferred segment also contains the leaf rust resistance gene *Lr35*. To identify *Sr39*, the markers *Sr39#22r* and

Sr39#50s were used. The first marker revealed a fragment of 820 bp in all samples, which did not correspond to the diagnostic fragment of 487 bp. A similar situation was observed for the second marker: with a declared fragment of 167 bp, products of 240 and 280 bp were amplified in the samples. At this stage of research, we can assume that the chromosomal region containing the annealing site of these primers is missing. Later, when we obtain a control line with the *Sr39* gene, repeat PCR analysis will allow us to confirm this assumption.

The stem rust resistance gene *Sr47* was transferred from *Aegilops speltoides* by homeologous recombination using the *ph1b* mutant into durum wheat, resulting in the DAS15 line. We tested for the presence of the *Sr47* gene, but without a control, as was the case with *Sr39*. A pronounced 109-bp product was observed in most accessions, except for accessions 14-16i, 19-16i, 25-16i, 31-16i, and 44-16i, which did not contain it. The researchers note that when using Xgwm501, a saturated fragment of 109 bp in length indicates the presence of *Ae. speltoides* chromatin, while the absence of the fragment or its lesser expression may indicate a segment of the wheat chromosome [30].

As a result of molecular screening, both single *Lr* and *Sr* genes and their pyramid were detected in the studied spring common wheat lines (Table 3). The greatest number of identified genes was found in line 53-16i (*Lr26, Lr37, Sr17, Sr22, Sr36, Sr47*), while in lines 1-16i, 28-16i, 49-16i, 61-16i the studied *Lr* genes were not detected. Considering the high resistance of the latter genotypes in the conditions of the Altai Territory and the southern forest-steppe of Western Siberia, there is a high probability of the presence of other effective resistance genes (e.g. *Lr35, Sr32, Sr39, Sr40*) or new resistance genes transferred from *Ae. speltoides*, *T. kiharae* or *T. migushovae*.

Table 3

Identification of *Lr* and Sr genes in spring common wheat lines from Arsenal collection

Variety/Line	*Lr*–genes			Sr–genes			
	*Lr*26	*Lr*34	*Lr*37	*Sr*17	*Sr*22	*Sr*36	*Sr*47
Altaiskaya-70	–	–	–	–	–	–	–
1–16i	–	–	–	–	+	+	+
5–16i	+	–	+	–	+	+	+
6–16i	+	–	–	–	+	+	+
14–16i	+	–	–	–	+	+	–
17–16i	+	–	+	–	–	+	+
19–16i	+	–	–	–	+	+	–
20–16i	+	–	–	+	+	+	+
21–16i	+	–	–	–	–	+	+
25–16i	+	–	–	–	+	–	–
28–16i	–	–	–	–	+	+	+
30–16i	+	–	+	–	+	+	+
31–16i	+	–	+	–	+	+	–
34–16i	–	+	+	+	–	+	+

Ending tabl. 3

Variety/Line	*Lr*–genes			Sr–genes			
	*Lr*26	*Lr*34	*Lr*37	*Sr*17	*Sr*22	*Sr*36	*Sr*47
36–16i	+	–	–	–	+	+	+
37–16i	+	–	–	–	+	+	+
40–16i	–	+	–	–	–	+	+
44–16i	+	–	+	+	+	+	–
45–16i	+	–	+	–	+	+	+
48–16i	+	–	+	–	+	+	+
49–16i	–	–	–	–	+	+	+
53–16i	+	–	+	+	+	+	+
57–16i	–	–	+	–	+	+	+
60–16i	–	–	+	–	+	+	+
61–16i	–	–	–	–	+	+	+
135/10i	–	+	–	–	+	–	+

Source: compiled by V.A. Petin.

Conclusion

The presence of a wide range of leaf and stem rust resistance genes in wheat lines from Arsenal collection was identified, indicating the donor properties of these lines and the possibility of their effective use in MAS to overcome the genetic uniformity of local varieties in terms of resistance to rust diseases and address the phytosanitary situation in the region.

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Идентификация генов устойчивости к листовой и стеблевой ржавчине у селекционных линий яровой мягкой пшеницы из вторичного генофонда коллекции «Арсенал»

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Аннотация. Листовая (*Puccinia triticina* Eriks.) и стеблевая (*Puccinia graminis* f. sp. tritici) ржавчины являются основными причинами снижения урожайности пшеницы в России и за рубежом. Эпифитотии этих болезней приводят к значительным экономическим потерям. Наблюдается усиление давления со стороны новых, более агрессивных рас патогенов. Стратегия селекции, направленная на защиту сортов пшеницы от этих болезней, является крайне актуальной и приоритетной. Цель исследования — идентификация эффективных и частично эффективных генов устойчивости к листовой (*Lr19, Lr24, Lr26, Lr29, Lr34, Lr37*) и стеблевой (*Sr17, Sr22, Sr36, Sr39, Sr47*) ржавчине в Приобской лесостепи Алтайского

края для дальнейшей разработки комплекса селекционных мероприятий, направленных на создание новых сортов яровой пшеницы с устойчивостью к ржавчинным болезням в местных условиях с использованием современных методов молекулярно-маркерной селекции. Материалом для исследования служили 25 перспективных линий яровой мягкой пшеницы из вторичного генофонда коллекции «Арсенал» (ФИЦ «Немчиновка») с групповой устойчивостью к листостебельным болезням, имеющие в родословной генетический материал видов *Aegilops speltoides*, *Ae. triuncialis*, *Triticum kiharae*, *Secale cereale* и *T. migushovae*. Молекулярный анализ позволил определить эффективные гены *Lr* у 80 %, а гены *Sr* — у всех протестированных образцов. Наибольшее число идентифицированных генов (5–6) выявлено у линий: 5–16i, 20–16i, 34–16i, 44–16i, 45–16i, 48–16i, 53–16i, а наименьшее (2–3) — 1–16i, 14–16i, 19–16i, 21–16i, 25–16i, 40–16i, 49–16i, 61–16i, 135/10i. У образцов 1–16i, 28–16i, 49–16i, 61–16i искомых генов *Lr* не обнаружено. Результаты исследований показали наличие широкого спектра генов устойчивости к листовой и стеблевой ржавчине, что указывает на донорские свойства линий коллекции «Арсенал» и возможность их эффективного использования в маркер-ассоциированной селекции при создании сортов пшеницы, устойчивых к ржавчинным болезням.

Ключевые слова: ДНК-маркёры, болезни растений, сельскохозяйственная генетика, грибные патогены, *Lr*-гены, *Sr*-гены, маркер-ассоциированная селекция

Вклад авторов: Петин В.А. — проведение опытов, написание текста рукописи; Лепехов С.Б. — разработка концепции, проверка и редактирование текста рукописи; Лапочкина И.Ф., Гайнуллин Н.Р. — обсуждение и утверждение окончательного варианта рукописи.

Финансирование. Работа выполнена при финансовой поддержке проекта Министерства образования и науки Российской Федерации «Развитие селекционно-семеноводческого центра в сфере зерновых и зернобобовых культур — структурного подразделения Федерального государственного бюджетного научного учреждения „Федеральный Алтайский научный центр агробиотехнологий“» Соглашение № 075-15-2025-186 от 18 апреля 2025 г.

Заявления о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов.

История статьи: поступила в редакцию 19 июня 2025 г., принята к публикации 15 октября 2025 г.

Для цитирования: *Петин В.А., Лепехов С.Б., Лапочкина И.Ф., Гайнуллин Н.Р.* Идентификация генов устойчивости к листовой и стеблевой ржавчине у селекционных линий яровой мягкой пшеницы из вторичного генофонда коллекции «Арсенал» // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2025. Т. 20. № 4. 577–588. doi: 10.22363/2312-797X-2025-20-4-577-588 EDN: CABXNN