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ENLARGEMENT OF GENETIC DIVERSITY OF SPRING BREAD WHEAT RESISTANCE TO LEAF RUST (*Puccinia triticina* Eriks.) IN LOWER VOLGA REGION

E.I. GULTYAEVA[1], S.N. SIBIKEEV[2], A.E. DRUZHIN[2], E.L. SHAYDAYUK[1]

[1]*All-Russian Research Institute of Plant Protection,* 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail eigultyaeva@gmail.com (✉ corresponding author), eshaydayuk@bk.ru;
[2]*Agricultural Research Institute for South-East Regions,* 7, ul. Tulaikova, Saratov 410010 Russia, e-mail sibikeev_sergey@mail.ru, alex_druzhin@mail.ru
ORCID:
Gultyaeva E.I. orcid.org/0000-0001-7948-0307
Sibikeev S.N orcid.org/0000-0001-8324-9765
Druzhin A.E. orcid.org/0000-0002-3968-2470
Shaydayuk E.L. orcid.org/0000-0003-3266-6272
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Abstract

Leaf rust (*Puccinia triticina* Eriks.) is the significant disease of winter and spring wheat in Russia. In the Volga region, the epiphytoties of this disease are observed on average once per three to four years. The genetic protection of wheat from leaf rust is a priority. Its successful practical implementation is possible only by the increasing of the genetic diversity of the commercial wheat cultivars, particularly by effective combinations of the known genes for resistance or use in the hybridization donors of new *Lr*-genes, from species of genera *Triticum* and *Aegilops*. On the basis of highly productive and adaptive spring bread wheat cultivars (Prokhorovka, Saratovskaya 29, Saratovskaya 55, Saratovskaya 68, Saratovskaya 70, Saratovskaya 73, Saratovskaya 74, L503, L505, Dobrynya, Favorite, Belyanka, Voevoda of Saratov Breeding Center) and alien species the introgression lines are derived which possess high resistance to leaf rust and are promising as breeding material. It was of interest to study the genetic determination of leaf rust resistance in these new lines and to evaluate their effect on the variability of *P. triticina* population for virulence in Saratov region. A total of 42 introgression lines were investigated. Donors of alien *Lr*-genes were the lines of cultivar Thahcher with *Lr24*, *Lr29*, *Lr36* genes, and cultivars with *Lr37* gene, and also species *Triticum dicoccum*, *T. kiharae*, *T. timopheevii*, *T. durum*, *T. petropavloskyi*, *T. persicum*, *Aegilops tauschii*, *Secale sereale* and *Agropyron elongatum*. Leaf rust resistance genes (*Lr*-genes) were identified by phytopathological tests and DNA markers. The studied lines of spring bread wheat showed high genetic diversity for leaf rust resistance. Among them, we have identified the carriers of known *Lr*-genes which have not yet been used in breeding of spring bread wheat in Russia (L4 with *Lr29*), and also the carriers of presumably new *Lr*-genes transferred from *T. durum* (L8, L39 for *Lr19* + *LrTdur*, L25, L19, L11 for *Lr10* + *Lr19* + *LrTdur*), *T. persicum* (L38 for *Lr19* + *LrT.pers*), *T. timopheevii* (L49 for *Lr10* + *LrT.tim*), *Ae. tauschii* (L6 for *Lr19* + *LrA.tau*), and *T. kiharae* (L33 for *Lr3* + *Lr19* + *LrT.kh*). Lines L10, L13, L46, L24, L48, L5 and L9 have the effective combination of *Lr19* + *Lr26* genes, L2, L28 L29 of *Lr10* + *Lr19* + *Lr26*, L42 of *Lr19* + *Lr37*, L44 of *Lr19* + *Lr26* + *Lr39*, L3 of *Lr19* + *Lr37* + *Lr6Agi*, L4 of *Lr19* + *Lr6Agi*, L7 of *Lr10* + *Lr26* + *Lr6Agi*, L45 of *Lr10* + *Lr19* + *Lr39* + *Lr6Agi*, and L40 of *Lr10* + *Lr39* + *Lr6Agi*. The virulence of the pathogen of the Saratov population was characterized in 2017 and 2018. The samples were collected from susceptible wheat cultivars which grew together with the studied introgression lines. The *Lr9*, *Lr24*, *Lr28*, *Lr29*, *Lr41*, *Lr42*, *Lr45*, *Lr47*, *Lr50*, *Lr51*, *Lr53*, and *Lr6Agi* genes (infection type 0 and 0;) were highly effective. Lines with *Lr28*, *Lr29*, *Lr41, Lr51*, and *Lr6Agi* genes also showed high resistance under field conditions. Thus, all these genes are perspective for breeding in the Volga region to expand genetic diversity of wheat cultivars. The presence of the isolates virulent to Tc*Lr19* lines was moderate, 16 % in 2017 and 20 % in 2018. All isolates virulent to *Lr19* were avirulent to *Lr26*, which confirms the effectiveness of this combination of *Lr*-genes in plant protection from leaf rust. This research resulted in a novel breeding material that combines resistance to leaf rust with adaptability to environmental factors, productivity and grain quality. Its distinctive feature is new donors of resistance involved from related species. Among tested lines there are donors which effectively combine either known *Lr*-genes or known and supposedly

new alien *Lr*-genes. The linkage of *Lr19*, *Lr26*, *Lr34*, *Lr37* genes with effective genes for resistance to other diseases, in particular to stem rust, will determine the resistance of new lines to a complex of diseases.

Keywords: *Puccinia triticina*, virulence, avirulence, *Triticum aestivum*, introgression lines, *Lr*-genes

Brown (leaf) rust (*Puccinia triticina* Erikss.) is a disease of common wheat with significant economic impact in many countries, including the Russian Federation. In the Volga region, the disease occurs almost annually, with epiphytoties observed on average once every three to four years. Crop losses can reach 20-30% (35% under irrigation), while the content of protein and gluten in the grain is significantly lower [1-3]. An analysis of the chronology of epiphytoties suggests that in the Volga region losses from leaf rust have recently become severer than in the first half of the 20th century [4]. Protecting bread wheat from this disease is becoming a priority. Improvement of genetic diversity of locally bred, highly productive and adapted to Volga region spring bread wheat varieties via involvement of *Triticum* or *Aegilops* species as donors or through a combination of known *Lr* genes is deemed most effective.

The first attempts to produce wheat varieties by introgressive hybridization with closely related species were made in Lower Volga Region in the first half of the 20th century by crossing bread wheat (*Triticum aestivum* L.) with *Triticum durum* Desf. [5]. As a result, spring bread wheat varieties Sarroza, Sarrubra, Albosar, Blansar were obtained, of which Sarrubra was regionalized in 1931 and occupied about 1.3 million ha in the early 1940s [6]. Later, species of the genera *Triticum*, the *T. durum*, *T. dicoccum* Schuebl., *T. dicocoides* (Koern. ex Aschers. et Graebn) Schweinf., and *Agropyron*, the *Ag. intermedium* (Host.) Beauv., *Ag. longatum* (Host.) P.B., as well as *Secale cereale* L. were involved to expand the regional genetic diversity of common wheat varieties in the region. The resultant varieties were L503, L505, Dobrynya (with genetic material from *Ag. Elongatum*), Belyanka (*Ag. intermedium*), Favorit, Voevoda (a combination of genetic material from *Ag. intermedium* and hard wheat variety Krasnokutka 10), Lebedushka (genetic material from Ag. *elongatum* and Ag. *intermedium*), Prokhorovka, Yugo-vostochnaya 2 (*Secale cereale*) [6].

Improving plant protection by increasing genetic diversity of highly productive wheat varieties via donors of new *Lr* genes or an effective combination of known *Lr* genes allows the epiphytotic situation with brown rust to be stabilized [2]. The genetic diversity of *Lr* genes among spring common wheat hybrids that are highly resistant to brown rust is an extremely important fundamental and practical issue.

The knowledge on the virulence-based genetic structure of a pathogen population is a background of the advanced breeding for crop resistance. It allows researchers to optimize strategies for using new resistance donors to control the phytosanitary situation [2, 3]. The *P. triticina* virulence in the Volga Region have been studied since 1970 [7]. Long-term observations show that the Lower Volga population of brown rust pathogen is evolutionarily active, and its virulence is increasing [8, 9]. This is primarily caused by the use of new genetically protected wheat varieties, as well as the fact that the territory of the Lower Volga Region is subjected to the inoculum drift from the North Caucasus, from Western Europe and Central Asia [10].

In this paper, we give the first results on the resistance gene diversity of promising spring bread wheat lines in the conditions of Lower Volga Region. Effective combinations of resistance genes, carrier lines of new unidentified *Lr* genes introgressed from durum wheat varieties, as well as the structure of the brown rust pathogen population in the Saratov Region are determined.

Our objective was to identify genetic determinants of brown rust resistance in new introgression lines of spring common wheat, to investigate changes in the composition of the present brown rust pathogen populations and to estimate prospects of using the obtained set of lines carrying *Lr* genes in breeding for brown rust resistance in the Volga Region.

Materials and methods. The promising introgression lines of bread wheat (n = 42) which showed high resistance to brown rust in the Lower Volga region in 2014-2018 we tested. Spring common wheat varieties Saratovskaya 68, Saratovskaya 70, Saratovskaya 73, Saratovskaya 74, Favorit, Dobrynya, Belyanka, Voevoda, L503, L505, Prokhorovka were widely used as recurrent parents. To increase genetic diversity on brown rust resistance, these varieties were crossed with the carriers of alien effective genes (*Lr24*, *Lr29*, *Lr36*, *Lr37*, etc.), with brown rust resistant specimens of *T. durum*, *T. dicoccum* (Schrank) Schuebl., *T. persicum* (Percival) Vavilov., *T. timopheevii* Zhuk., *T. kiharae* Dorof. et Migusch., *Aegilops squarrosa* L. (= *Ae. tauschii* L.) [11, 12] and with the susceptible species *T. petropavloskyi* Udacz et. Migusch.

Brown rust resistance of the introgression lines was assessed in lab tests on seedlings (1st leaf phase) and in field trials (plants in the phase of milk and milk-wax ripeness; Agricultural Research Institute for the South-East Regions, (ARISER), natural infectious). Seedlings were inoculated with four geographically distant populations of *P. triticina* (Saratov, Chelyabinsk, Krasnodar, Dagestan) sampled in 2018, and with three test clones marked by the virulence for *Lr9*, *Lr19* and *Lr26* carriers.

The tested wheat lines were sown in pots with soil. At the 1st leaf (days 10-14), the seedlings were inoculated with an aqueous suspension of pathogen spores (1×10^6/ml) with Tween 80 detergent added. The infected plants were grown in a moist chamber in the dark for 12-14 hours, and then transferred to a climate chamber (Versatille Environmental Test Chamber MLR-352H, SANYO Electric Co., Ltd, Japan) (22 °C, 75% humidity). On day 10 the lesions were recorded as per Mains and Jackson scale [13]: 0 — no symptoms, 0; — necrosis without pustules, 1 — very small pustules surrounded by necrosis, 2 — medium sized pustules surrounded by necrosis or chlorosis, 3 — medium sized pustules without necrosis, 4 — large pustules without necrosis, X — different types of pustules on the same leaf, chloroses and necrosis are present. Plants with a type infection of 0, 0;, 1, and 2 were classified as resistant, 3, 4, X as susceptible.

Molecular markers for identification of 22 *Lr* genes were WR003 (*Lr1*) [14], Xmwg798 (*Lr3*) [15], SCS5 (*Lr9*) [16)], Fi.2245/Lr10-6/r2 (*Lr10*) [17], SCS265 (*Lr19*) [18], STS638 (*Lr20*) [19], Lr21L/R (*Lr21*) (https://maswheat.ucdavis.edu/protocols/Lr21/index.htm), WMS296 (*Lr22a*) [20], Sr24#12, Sr24#50 (*Lr24*) [21], Lr25F20/R19 (*Lr25*) (https://maswheat.ucdavis.edu/protocols/Lr25/index.htm), SCM9 (*Lr26*) [22], SCS421 (*Lr28*) [23], Lr29F24 (*Lr29*) [24], csLV34 (*Lr34*) [25], Sr39=22 (*Lr35*) [26], Ventriup/LN2 (*Lr37*) [27], GDM35 (*Lr41*) [28], marker for *Lr47* [29], WMS382, GDM87 (*Lr50*) (https://maswheat.ucdavis.edu/protocols/Lr50/index.htm), cfd1 (*Lr53*) [30], S13-R16 (*Lr66*) [31], J09/1_pr1,4a (*LrAgi*) [32, 33]. DNA from wheat plants was extracted by the Dorokhov and Kloke method [34].

The virulence of the Saratov population *P. triticina* was analyzed in 2017-2018. The inoculum was collected in the ARISER experimental field. Reproduction of population samples and obtaining monopustular isolates were performed by laboratory cultivation method [35]. Virulence of the pathogen and line resistance to brown rust was studied on the 1st leaf wheat seedlings as per description [36]. In 35 isogenic Thatcher lines and wheat varieties with genes *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*,

Lr15, *Lr16*, *Lr17*, *Lr18*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr26*, *Lr28*, *Lr29*, *Lr30*, *Lr39(=41)*, *Lr42*, *Lr44*, *Lr45*, *Lr47*, *Lr48*, *Lr49*, *Lr51*, *Lr53*, *Lr57* and *Lr6Agi* evaluated the resistance to the combined sample of Saratov pathogen population. The racial composition of the pathogen and the frequency of virulence to 20 differentiator lines were determined using monopuscular isolates. Phenotypes were identified by the North American nomenclature [37], based on the determination of virulence for groups of Tc*Lr* lines. In this paper, the following sequence of Tc*Lr* lines was used (by the set of *Lr* genes): 1 — *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*; 2 — *Lr9*, *Lr16*, *Lr24*, *Lr26*; 3 — *Lr3ka*, *Lr11*, *Lr17*, *Lr30*; 4 — *Lr2b*, *Lr3bg*, *Lr14a*, *Lr14b*; 5 — *Lr15*, *Lr18*, *Lr19*, *Lr20*. The literal code of phenotypes and virulence frequency was received via Virulence Analysis Tool (VAT) software (https://en-lifesci.tau.ac.il/profile/kosman/vat).

Results. Table 1 gives the characterization of the infectious material virulence, and Table 2 comprises the list of markers for identification of pathogen resistance genes.

1. Characterization of *Puccinia triticina* Erikss. virulence to Thatcher lines used in testing resistance of the spring bread wheat introgression lines to the pathogen

Populations and isolates	Origin	Virulence	Avirulence
		to Thatcher lines carrying *Lr* genes	
Test-clone1	Chelyabinsk Province, 2017	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr30*	*Lr19*, *Lr23*, *Lr24*, *Lr26*, *Lr28*, *Lr29*, *Lr44*
Test-clone2	Tambov Province, 2016	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr14a*, *Lr14b*, *Lr15*, *Lr17*, *Lr18*, *Lr19*, *Lr20*, *Lr30*, *Lr44*	*Lr9*, *Lr11*, *Lr16*, *Lr23*, *Lr24*, *Lr26*, *Lr28*, *Lr29*
Test-clone 3	Krasnodarskii Krai, 2017	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr17*, *Lr18*, *Lr20*, *Lr23*, *Lr6*, *Lr30*, *Lr44*	*Lr9*, *Lr16*, *Lr19*, *Lr24*, *Lr28*, *Lr29*
Pop _Sar	Saratov Province, 2018	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr14b*, *Lr15*, *Lr19*, *Lr20*, *Lr23*, *Lr26, Lr30*	*Lr9*, *Lr24*, *Lr28*, *Lr29*, *Lr44*
Pop_Kr	Krasnodarskii Krai, 2018	*Lr1*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr16*, *Lr17*, *Lr18*, *Lr23*, *Lr26*, *Lr30*, *Lr44*	*Lr9*, *Lr2a*, *Lr15*, *Lr19*, *Lr20*, *Lr24*, *Lr28*, *Lr29*
Pop _Chel	Chelyabinsk Province., 2018 год	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr30*	*Lr19*, *Lr23*, *Lr24*, *Lr26*, *Lr28*, *Lr29*, *Lr44*
Pop_Dag	The Republic of Dagestan, 2018	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr23*, *Lr26*, *Lr30*, *Lr44*	*Lr9*, *Lr19*, *Lr24*, *Lr28*, *Lr29*

2. PCR markers used to determine *Lr genes*

Gene	Marker	Nucleotide sequence 5′→3′	Size, bp	Referencce
Lr1	WR003F	GGGACAGAGACCTTGGTGGA	760	Qiu et al., 2007
	WR003R	GACGATGATGATTTGCTGCTGG		
Lr3	Xmwg798F	GGCTGTCTACATCTTCTGCA	365	Herrera-Foessel et al., 2007
	Xmwg798R	CAAGTGTTGAGAAGGAGAGT		
Lr9	SCS5F	TGCGCCCTTCAAAGGAAG	550	Gupta et al., 2005
	SCS5R	TGCGCCCTTCTGAACTGTAT		
Lr10	Fi.2245	GTGTAATGCATGCAGGTTCC	310	Chelkowski et al., 2008
	Lr10-6/r2	AGGTGTGAGTGAGTTATGTT		
Lr19	SCS265 F	GGCGGATAAGCAGAGCAGAG	512	Gupta et al., 2006
	SCS265 R	GGCGGATAAGTGGGTTATGG		
Lr20	STS638-L	ACAGCGATGAAGCAATGAAA	542	Neu et al., 2002
	STS638-R	GTCCAGTTGGTTGATGGAAT		
Lr21	Lr21L	CGCTTTTACCGAGATTGGTC	669	https://maswheat.ucdavis.edu/
	Lr21R	TCTGGTATCTCACGAAGCCTT		
Lr22a	WMS296F	AATTCAACCTACCAATCTCTG	131	Hiebert et al., 2007
	WMS296R	GCCTAATAAACTGAAAACGAG	121	
Lr24	Sr24 12F	CACCCGTGACATGCTCGTA	550	Mago et al., 2005
	Sr24 12R	AACAGGAAATGAGCAACGATGT		
Lr25	Lr25F20	CCACCCAGAGTATACCAGAG	1800	https://maswheat.ucdavis.edu/
	Lr25R19	CCACCCAGAGCTCATAGAA		
Lr28	SCS421F	ACAAGGTAAGTCTCCAACCA	570	Cherukuri et al., 2005
	SCS421R	AGTCGACCG AGATTTTAACC		

Continued Table 2

Lr29	Lr29F24F	GTGACCTCAGGCAATGCACACAGT	900	Procunier et al., 1995
	Lr29F24R	GTGACCTCAGAACCGATGTCCATC		
	SCM9F	TGACAACCC CCTTTCCCTCGT	207	Weng et al., 2007
	SCM9R	TCATCGACGCTAAGGAGGACCC		
Lr34	csLV34F	GTTGGTTAAGACTGGTGATGG	150	Lagudah et al., 2006
	csLV34F	TGCTTGCTATTGCTGAATAGT		
Lr35	Sr39=22F	AGAGAAGATAAGCAGTAAACATG	800	Mago et al., 2009
	Sr39=22R	TGCTGTCATGAGAGGAACTCTG		
Lr37	Venttriup	AGGGGCTACTGACCAAGGCT	259	Helguera et al., 2003
	LN2	TGCAGCTACAGCAGTATGTACACAAAA		
Lr39=Lr41	GDM 35F	CCTGCTCTGCCCTAGATACG	190	Pestsova et al., 2000
	GDM 35R	ATGTGAATGTGATGCATGCA		
Lr47	PS10F	GCTGATGACCCTGACCGG	282	Helguera et al., 2000
	PS10R	TCTTCATGCCCGGTCGGGT		
Lr50	WMS382-F	GTCAGATAACGCCGTCCAAT	139	https://maswheat.ucdav-is.edu/protocols/Lr50/index.htm
	WMS382-R	CTACGTGCACCACCATTTTG		
	GDM87F	AATAATGTGGCAGACAGTCTTGG	110	
	GDM87R	CCAAGCCCCAATCTCTCTCT		
Lr53	cfd1F	ACCAAAGAACTTGCCTGGTG	225	Dadkhodaie et al., 2010
	cfd1R	AAGCCTGACCTAGCCCAAAT		
Lr66	S13-R16F	GGTGAACGCTAAACCCAGGTAACC	695	Marais et al., 2010
	S13-R16R	CAACCTGGGAAGATGCTGAG		
LrAgi	J09/1	TCTAGTCTGTACATGGGGGC		Schachermayr et al., 1995
	Pr1, 4a	Confidential information		Sibikeev et al., 2018

A characteristic feature of the Saratov breeding school in production of wheat varieties is the continuity and improvement of the local highly adapted gene pool with new genetic material [38]. Spring bread wheat varieties that we used as a recurrent parent belong to the group of highly productive and widely cultivated in the Lower Volga and other Russian regions [39]. These varieties differ significantly in their resistance to brown rust. The group of varieties of the Saratovskaya brand (Saratovskaya 29, Saratovskaya 55, Saratovskaya 68, Saratovskaya 70, Saratovskaya 73, Saratovskaya 74) and the variety Prokhorovka are highly susceptible. PCR analysis showed that most of them have an ineffective *Lr10* gene (with the exception of Saratovskaya 55 and Saratovskaya 70), and Prokhorovka variety additionally carry *Lr26* gene (Table 3).

Lr19 gene protects varieties L503, L505 and Dobrynya. *Lr10* gene is also identified in varieties L503 and L505. Seedlings and adult plants of these varieties showed resistance to pathogen populations, avirulent to lines and cultivars with *Lr19*, and susceptibility to virulent ones (see Table 3). The degree of the damage in the field conditions of the Lower Volga Region varied from 0 to 20%, because of different abundance of isolates that are virulent for plants with the *Lr19* gene, since the area under cultivars carrying this resistance gene has been reducing. In Russia, the first varieties with the *Lr19* gene began to be cultivated since the late 1980s in the Volga Region. When their crop areas in the mid-1990s exceeded 100 thousand ha, the protective effect of *Lr19* was overcome [4]. Currently, virulence to carriers of this gene is recorded both whithin and beyond the regions of cultivation varieties with *Lr19* [40, 41].

Varieties Belyanka, Voevoda, Favorit are the carriers of the Lr6Agi gene which is transferred from the wheatgrass *Elytrigia intermedia* (Host) Nevski and is not identical to the gene included in the gene symbol catalog. These varieties are characterized by high juvenile resistance over a long period of their regionalization [32].

To date, 77 *Lr* genes have been identified worldwide and over 50% of them are alien [42]. Their sources are species *Ae. tauschii* carrying *Lr21*(=*Lr40*), *Lr22a*, *Lr32*, *Lr39*(=*Lr41*), *Ae. umbellulata* (*Lr9*, *Lr76*), *Ae. speltoides* (*Lr28*, *Lr35*, *Lr36*, *Lr47*, *Lr51, Lr66*), *Ae. ventricosa* (*Lr37*), *Ae. kotschyi* (*Lr54*), *Ae. sharonensis* (*Lr56*), *Ae. geneculata* (*Lr57*), *Ae. triuncialis* (*Lr58*), *Ae. peregrina* (*Lr59*), *Ae. neglecta* (*Lr62*), *T. spelta* (*Lr44, Lr71*), *S. cereale* (*Lr25*, *Lr26*, *Lr45*), *T. timo-*

3. Resistance of ARISER spring bread wheat introgression lines to brown (leaf) rust and identified *Lr* genes (experimental field of ARISER, Saratov, 2016-2018)

Line	Pedigree	SH	FD, %/score	Damage to seedlings, score							*Lr* genes
				1	2	3	4	5	6	7	
			Group I								
L3	Milan/Prinia*4//Dobr/3/Fav	*Aegilops ventricosa*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr37* + *Lr6Agi*
L42	Dobr/Trident//Dobr/3/Dobr/4/Dobr	*Aegilops ventricosa*	0/0;	0	0	2–	0	3	0	1-2;	*Lr19* + *Lr37*
L10	L164/Prokh//L164/Dobr *Lr9*	*Ae. umbellulata*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L4	Dobr*2//L2033/Bel/3/Dobr *Lr24*	*Ag. elongatum*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr6Agi*
L52	S70/Tc*Lr29**4//S70	*Ag. elongatum*	0-2/0; and 1	0	0	0	0	0	0	0	*Lr29*
L30	L503 Tc*Lr36*//L503/3/L503	*Ae. speltoides*	0-2/1	0	3	0-1;	0	0	0	0-1	*Lr10* + *Lr19*
			Group II								
L8	L164/Sar. zol//S68	*Triticum durum*	0-5/0 and 3	0	0	0	0	0	0	0	*Lr19* + *LrTdur*
L25	S58*2//Zol. volna/3/S68	*Triticum durum*	0/0;	0	0	0	0	0	0	0	*Lr10* + *Lr19* + *LrTdur*
L39	Dobr/Zol. volna//Dobr/3/Dobr	*Triticum durum*	0/0;	0	0-1	0	0	0	0	0	*Lr19* + *LrTdur*
L43	Dobr*4/Nik	*Triticum durum*	0/0;	0	3	0	0	0	0	0	*Lr19*
L13	Dobr*3//Nik	*Triticum durum*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L19	Dobr*4/Nik/Grekum S2193 L1314/2 16	*Triticum durum*	0-5/0; and 3	0	0	0	0	0	0	0	*Lr10* + *Lr19* + *LrTdur*
L2	L505/L164/4/L503//Trap#1/Bow/3/L503/5/L505/6/Al32	*Triticum durum*	0/0;	0	0	0-1;	0	0	0	0	*Lr10* + *Lr19* + *Lr26*
L11	L505/L164//Prokh	*Triticum durum*	0/0;	0	0-1;	0	0	0	0	0	*Lr10* + *Lr19* + *LrTdur*
L46	Prokh/L164//Prokh/3/L164/4/L164	*Triticum durum*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L38	Dobr/*T. persicum*//Dobr	*T. persicum*	0/0;	0	0-1	0	0	0	0	0	*Lr19* + *LrTpers*
L24	S74/*T. dicoccum* k7507//S73	*T. dicoccum*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L28	S74/*T. dicoccum* k7507//S73/3/S73 L1504/2 16	*T. dicoccum*	0-5/0 and 3	0	0	1-2;	0	0	0	0-1-2;	*Lr10* + *Lr19* + *Lr26*
L29	S74/*T. dicoccum* k7507//S73/3/S73	*T. dicoccum*	0/0;	0	0-1	0	0	0	0	0-1	*Lr10* + *Lr19* + *Lr26*
L47	S74/*T. dicoccum* k7507//S73/3/S73	*T. dicoccum*	0/0;	0	0	3	0	0-2	0	3	*Lr10* + *Lr26*
L48	S74/*T. dicoccum* k7507//S73/3/S73	*T. dicoccum*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L5	S55*5/*T. dicoccoides*// Dobr *Lr9*	*T. dicoccoides* + *Lr9*	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L49	S68/*T. timopheevii**4//Dobr	*T. timopheevii*	0/0;	0	0	0	0	0	0	0	*Lr10* + *Lr28*=*LrTtim?*
			Group III								
L6	Croc/*Ae. squarrosa* (205)//Weaver/3/*3 L505	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0;	0	0	0	0	0	*Lr19* + *LrAtau?*
L7	Bel/3/Croc/*Ae. squarrosa* (205)//Weaver/4/Bel	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr10* + *Lr26* + *Lr6Agi*
L9	Croc/*Ae. squarrosa*//Weaver/3/*3 L505	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26*
L20	Croc/*Ae. squarrosa* (205)//Weaver/3/L505/4/Bel/5/Fav/6/S74	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr10* + *Lr6Agi*
L40	Dobr/3/Croc/*Ae. squarrosa* (205)//Weaver/4/Dobr	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr10* + *Lr39* + *Lr6Agi*
L44	L505/3/Croc/*Ae. squarrosa* (205)//Weaver/4/L505/5/S68	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr19* + *Lr26* + *Lr39*

Continued Table 3

L45	Dobr/3/Croc/*Ae. squarrosa* (205)//Weaver/4/Dobr/5/Dobr	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr10 + Lr19 + Lr39 + Lr6Agi*
L51	Croc/*Ae. squarrosa* (205)//Weaver/3/L505/4/Bel/5/Fav/6/Fav	*Ae. tauschii*(=*Ae. squarrosa*)	0/0;	0	0	0	0	0	0	0	*Lr6Agi*
			Group IV								
L21	Voev/*T. petropavloskyi*//Voev	*T. petropavloskyi*	50/3	2-3	0	3	3–	3	3	3	*Lr10*
L31	Voev/*T. petropavloskyi**3//Voev	*T. petropavloskyi*	0/0;	0	0	0	0	0	0	0	*Lr6Agi*
			Group V								
L17	S70/*T. kiharae*//Dobr/3/Dobr	*T. kiharae*	15/1 и 3	0	3	0	0	0	0	0	*Lr19*
L18	S68/*T. kiharae*//L503	*T. kiharae*	0/0;	0	0-1	0	0	0	0-1;	0	*Lr19 + Lr28=LrTkh?*
L22	S68/*T. kiharae*//S70/3/S68/4/S68	*T. kiharae*	0/0;	0	0-2, 3	0	0	0	0	1-2;	*Lr10 + Lr19*
L32	S68/*T. kiharae*//S70/3/S70/4/S70	*T. kiharae*	0/0;	0	3	0	0	0	0	0	*Lr19*
L33	S68/*T. kiharae*//Dobr/3/Dobr	*T. kiharae*	0/0;	0	0	0	0	0	0	0	*Lr3 + Lr19 + Lr28=LrTkh?*
L53	S68/*T. kiharae*//Dobr/3/Dobr/4/Dobr	*T. kiharae*	0/0;	0	3	0	0	0	0;	0	*Lr3 + Lr19*
L56	Viktoria 95/No. 1	*T. miguschovae*	0/0;	0	–	–	0	0-1;	2-3	0-1;	*Lr1 + Lr3 + Lr34*
L57	Viktoria 95/No. 1	*T. miguschovae*	0-5/0; and 1	0-1	0-1;	0-1;	0-1;	3	3	3	*Lr1 + Lr3 + Lr34*
L58	Viktoria 95/No. 1	*T. miguschovae*	0-20/0; and 3	0	0	3–	0-1;	3	3	3	
			RIA ES spring soft wheat varieties								
	Saratovskaya 29		70/3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	*Lr10*
	Saratovskaya 55		70/3-4	3	3	3	3-4	–	–	–	
	Saratovskaya 68		40/3	3-4	3-4	3-4	3-4	–	–	–	*Lr10*
	Saratovskaya 70		70/3-4	3-4	3-4	3-4	3-4	–	–	–	
	Saratovskaya 73		50/3	3	3	2	3	–	–	–	*Lr10*
	Saratovskaya 74		60/3	3	3	3	3	–	–	–	*Lr10*
	Favorit	*Agropyron intermedium*	0/0;	0	0	0	0	0	0	0	*Lr6Agi*
	Voevoda	*Ag. intermedium*	0/0;	0	0	0	0	0	0	0	*Lr6Agi*
	Belyanka	*Ag. intermedium*	0/0;	0	0	0	0	0	0	0	*Lr6Agi*
	Dobrynya	*Ag. elongatum*	15/2-3	0	3	0	0	0	0	0	*Lr19*
	L503	*Ag. elongatum*	15/2-3	0	3	0	0	0	0	0	*Lr10 Lr19*
	L505	*Ag. elongatum*	15/2-3	0	3	0	0	0	0	0	*Lr10 Lr19*
	Prokhorovka	*Secale sereale*	30/3	0	0	3	0 и 3	0	0	3	*Lr10 Lr26*

N o t e. SH — species involved in hybridization, FD — damage under field conditions; populations and isolates of the pathogen: 1 — Test-clone1, 2 — Test-clone2, 3 — Test-clone3, 4 — Pop_Sar, 5 — Pop_Kr, 6 — Pop_Chel, 7 — Pop_Dag (for description of *Puccinia triticina* Erikss. populations and isolates see Table 1). S29 — Saratovskaya 29, S68 — Saratovskaya 68, S70 — Saratovskaya 70, S73 — Saratovskaya 73, S74 — Saratovskaya 74, Fav — Favorit, Dobr — Dobrynya, Bel — Belyanka, Voev — Voevoda, Prokh — Prokhorovka, Sar. zol — Saratovskaya zolotistaya, Zol. volna — Zolotaya volna. 0 — no signs, 0; — necroses without pustules, 1 — very small pustules surrounded by necrosis, 2 — medium-sized pustules surrounded by necrosis or chlorosis, 3 — medium-sized pustules without necrosis, 4 — large pustules without necrosis. Scores 0, 0;, 1, 2 mean plant resistance, 3, 4 mean plant susceptibility [13]. Dashes mean that the sample was not tested.

pheevii (*Lr18*, *Lr50*), *Ag. elongatum* (*Lr19, Lr24, Lr29*), *Ag. intermedium* (*Lr38*), *T. dicoccoides* (*Lr33*, *Lr53*, *Lr64*), *T. durum* (*Lr23, Lr61*) и *T. monococcum* (*Lr63*). Genes *Lr9*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26*, *Lr28*, *Lr37*, and *Lr39* were transferred to commercial varieties of common wheat [43, 44)]. Some of these samples (Thatcher lines with genes *Lr9*, *Lr24*, *Lr36*; varieties Trident and Milan with *Lr37*) we used to increase the genetic diversity of highly productive spring bread wheat varieties grown in the Lower Volga Region. Along with the known *Lr* genes, we used samples of alien species, presumably carrying new resistance genes [10, 11].

Group I. Lines L3 and L42 (*Lr37*), L52 (*Lr29*), L4 (*Lr24*), L10 (*Lr9*) and L30 (*Lr36*) were obtained using donors of known *Lr* genes (see Table 3). Molecular markers confirmed the presence of the resistance gene *Lr37* of adult plants of lines L3 and L42 produced with the participation of Milan and Trident varieties as donors of this gene. Also, the *Lr19* gene transferred from the Dobrynya variety was identified in these lines, and the additional *Lr6Agi* gene from the Favorite variety was identified in L3. Both lines were highly resistant in the field conditions of the Saratov region. Seedlings of the L3 line carrying genes *Lr19* + *Lr37* +*Lr6Agi*, when inoculated with clone No. 2 virulent to *Lr19* (see Table 3), responded significantly higher (score 0) than a susceptible L42 line (*Lr19* + *Lr37*), and were moderately resistant (score 1-2) upon inoculation with the Dagestan population and clone No. 3 virulent to *Lr26*.

Only the *Lr29* gene was identified in the L52 line, obtained on the basis of the brown rust susceptible variety Saratovskaya 70 and the Tc*Lr29* line. Seedlings of the L52 line, as well as the initial isogenic line Tc*Lr29*, were highly resistant to all geographical populations and clones of the pathogen (reaction type 0). In the field conditions, their response varied from 0; to 1. Until now, *Lr29* the donor of which is *Ag. elongatum* has not been used in Russian and foreign breeding programs [12, 42].

In lines L4 and L10, which pedigrees involve Tc*Lr24* and Tc*Lr9*, we did not identify these genes. Molecular analysis determined *Lr19* inherited from the varieties Dobrynya and L503. An additional *Lr26* gene introgression from the Prokhorovka variety was detected in the L10 line, and *Lr6Agi* from the Belyanka cultivar was found in L4. The high resistance of L4 and L10 seedlings and adult plants indicates the effectiveness of the combinations *Lr19* + *Lr26* and *Lr19* + *Lr6Agi* genes in wheat protecting against brown rust in the Volga region.

L30 line with Tc*Lr36* in the pedigree showed susceptibility during seedling phase when infected with test clone No. 2 virulent to *Lr19* carriers. Molecular markers identified L30 as the carrier of *Lr19* + *Lr10* genes. Under field conditions, the L30 showed 1 point response that was lower than that of Tc*Lr19*, but higher than that of Tc*Lr36*, which may be due to the additive interaction of the *Lr10*, *Lr19*, and *Lr36* genes.

Group II. Tetraploid wheat species are believed to be more resistant to brown rust than diploids and hexaploids [45]. However, only a few *Lr* genes were moved from them to common wheat. *Lr23* introduced from *T. durum* is the most frequently transferred [12]. The *Lr23* gene lost its effectiveness in the Volga region in the late 1990s. However, under field conditions bread wheat varieties with this gene show different residual resistance effects. The varieties of durum wheat Saratovskaya zolotistaya, Zolotaya volna and Nik involved in L8, L25, L13, L19, L39 and L43 development, are resistant to brown rust in the Lower Volga Region [46]. The genetic control of their resistance to this disease is undisclosed. However, in the pedigree of the Zolotaya volna and Nick varieties, there is Saratovskaya zolotistaya with a type of reaction to the leaf rust pathogen 1.1+.

In our study, most of the introgression lines produced with the partici-

pation of durum wheat varieties were characterized by high resistance during period of seedlings as well as adult plants. The exception was the line L43 which was attacked by pathogen clone No. 2 virulent to Lr19. For L8 and L19, we noted a segregation on resistance to disease in the field, which indicates the heterogeneity of these lines and the need for further selection.

Lr19 gene was detected in all lines based on Dobrynya variety (L13, L39, L43, L19) and *T. durum* Zolotaya volna and Nik varieties (see Table 3). In L13, *Lr26* gene was also identified the combination of which with *Lr19* can determine high resistance of this line. L19 carries ineffective *Lr10* gene. The *Lr19* gene was also detected in L25, while its donors were not in the pedigree. *Lr10* gene the source of which was the Saratovskaya 68 variety was also detected in this line. A high resistance of seedlings and adult plants in lines L39, L19, and L25 indicates the presence of additional genetic material from *T. durum* along with translocation from *Ag. elongatum*.

Lines L2, L8, L11 and L46 were obtained with the participation of L164 = L504/Saratovskaya 57//L504. Their durum wheat-derived genetic material could be translocated from L164, in the pedigree of which there is Saratovskaya 57 variety resistant to brown rust. All lines of this group carry *Lr19*, which is consistent with the analysis of the L2 and L1 pedigrees in creation of which line L505 participated. *Lr10* was identified in L11 and L2. The *Lr26* gene was not inherited from the Prokhorovka cultivar. High resistance of its seedlings and adult plants suggests the presence of an additional *Lr* gene from Saratovskaya 57 durum wheat. In L2, the Lr26 gene of CIMMYT (International Maize and Wheat Improvement Center) line Trap#1/Bow was determined.

The gene combination *Lr10* + *Lr19* + *Lr26* in the L2 line leads to high juvenile and adult resistance to brown rust. As already noted, the *Lr19* gene was identified in L8 and L46, but its origin, as per the pedigrees, is not clear. Nevertheless, according to the pedigree, L46 may have *Lr26* from the Prokhorovka cultivar, as it was confirmed by molecular analysis. Therefore, L46 carries *Lr19* + *Lr26* combination. L8 showed high resistance to all leaf rust samples, which cannot be caused by the presence of only *Lr19*, therefore there is reason to assume the additional genes from durum wheat (*LrTdur*) of the Zolotaya volna and Saratovskaya 57 which has L164 in the pedigree. Two recessive brown rust resistance genes transmitted from durum wheat Saratovskaya 57 were previously identified in L164 [47].

Along with durum wheat, tetraploid species *T. persicum*, *T. dicoccoides*, *T. dicoccum* of similar genomic composition (AuAuBB), as well as *T. timopheevii* (GGAtAt) were used to improve genetic diversity of Saratov spring bread wheat varieties. In the L38 line based on Dobrynya cultivar and *T. persicum* sample, one *Lr19* gene was established using DNA markers. Moreover, this line was high resistant throughout the growing season, which indicates the presence of an additional *Lr* gene from *T. persicum*. The gene symbol catalog [42] does not contain information on genes moved to common wheat from this species; therefore, it can be assumed that the L38 line has a new *Lr* gene, which in combination with *Lr19* provides high protection against brown rust.

Most lines based on susceptible varieties Saratovskaya 74 and Saratovskaya 73 and sample *T. dicoccum* k-7507 (Iran), the L24, L28, L29, L47, and L48, contain a combination of the *Lr19* and *Lr26*. A similar combination was identified in the L5 line obtained with the participation of *T. dicoccoides*. The L28 line is heterogeneous on *Lr19*, which probably causes its segregation of brown rust resistance in field tests. In L28 and L29, the *Lr10* gene was also determined. The L47 line differed from these lines in susceptibility to test clone No. 3 virulent to *Lr26*. Molecular markers revealed in this line a combination of

two ineffective genes, *Lr10* + *Lr26*. Moreover, this line, like other lines involving *T. dicoccum*, was high resistant in field tests, which indicates the presence of additional *Lr* genes. It was previously shown that resistance to brown rust in *T. dicoccum* k-7507 is controlled by one dominant *Lr* gene [10]. The reason for the presence of genes from *Agropyron elongatum* (*Lr19*) and rye (*Lr26*) is unclear. However, the combination of *Lr* genes can cause a high resistance. The gene symbol catalog [42] describes two genes, *Lr53* and *Lr64*, translocated to common wheat from *T. dicoccum*. The line with *Lr53* in our long-term investigations was high juvenile resistant to all *P. triticina* Russian populations, including the Saratov one (score 0, 0;, 1). Two alleles of 320 bp and 375 bp were amplified by cfd1 marker in the *Lr53*-bearig positive control (Fig. 1), while one 275 bp allele was amplified in L5, L24, L29, L47, and L48 lines, which indicates the lack of *Lr53* [29].

L49 was obtained via hybridization of *T. timopheevii* and bread wheat varieties Saratovskaya 68 (*Lr10*) and Dobrynya (*Lr19*). Molecular analysis revealed *Lr10* and *Lr28* in this line, whereas *Lr19* gene of Dobrynya variety was not detected. The detection of the SCS421 marker, in our opinion, indicates the presence of the *T. timopheevii* (*LrTtim*) genetic material in the sample. We showed earlier [48] that this marker is not strictly specific to determine *Lr28* gene from *Ae. speltoides*, and is also present in samples of *T. timopheevii*.

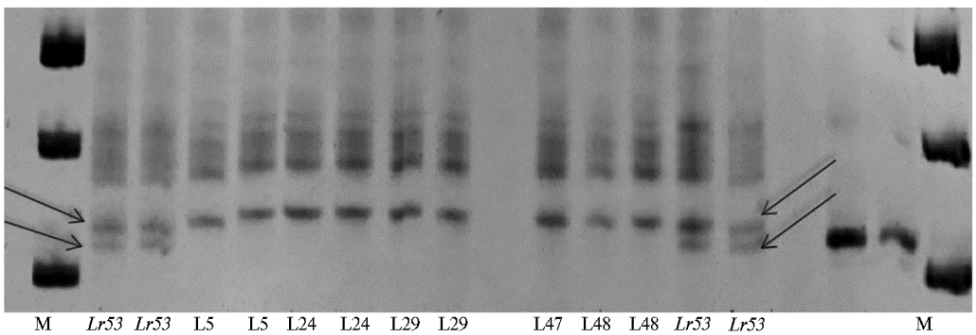

Fig. 1. PCR identification of cfd1 marker of *Lr53* in introgression lines (L) **of spring soft wheat (ARISER):** M — molecular weight marker (DNA length marker 50 bp, Diaem, Russia), *Lr53* — positive control (Tc*Lr53*). Arrows indicate 320 bp and 375 bp PCR products.

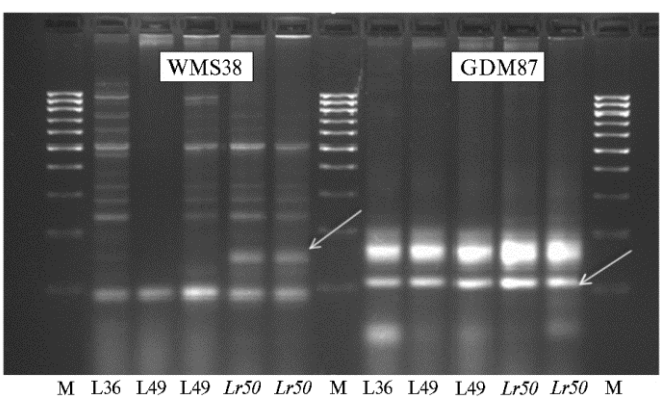

Fig. 2. PCR identification of microsatellite markers WMS382 and GDM87 of *Lr50* in introgression lines (L) **of spring bread wheat (ARISER):** M — molecular weight marker (DNA length marker 100 bp, Diaem, Russia), *Lr50* — positive control (line KS96WGRC36). Arrows indicate 139 bp (WMS382) and 110 bp (GDM87) PCR products.

In the gene symbol catalog [42], there are two genes, the *Lr18* and *Lr50*, moved to common wheat from *T. timopheevii*. The *Lr18* gene is ineffective in the Volga Region. Seedlings of the line with this gene are susceptible to brown leaf rust (score 3-4). A response of the line with *Lr50* upon inoculation with the Saratovskaya population of the pathogen varied from 0-1 to 2+ points and differed from that of L49. WMS382

marker of the *Lr50* gene is more closely linked to this gene (6.7 cM) than GDM87 (9.4 cM). The electrophoretic pattern we obtained indicated the absence of *Lr50* in this line (Fig. 2). The results for GDM87, which was detected in the L49 line and the L36 line, turned out to be false positive. Similar cases of inefficiency of this marker for screening *Lr50* are widely discussed in the literature (https://maswheat.ucdavis.edu/protocols/Lr50/index.htm), and therefore it is recommended for use in marker-assisted selection (MAS) only as optional to WMS382.

Group III. Diploid species *Ae. tauschii* is used worldwide to confer disease resistance and other economically valuable traits. In our work, synthetic amphidiploid Croc/*Ae. squarrosa* (205)//Weaver (CIMMYT) was involved to produce lines L6, L7, L9, L20, L40, L44, L45 and L51. This synthetic amphidiploid has a complex of economically valuable traits and is used in plant breeding in many countries [49, 50]. This group of lines did not have the *Lr21* and *Lr22a* genes transmitted from *Ae. taushcii*, while *Lr39*(= *Lr41*) was found in lines L40, L44, and L45 (Fig. 3). The *Lr19* gene was inherited by lines L6, L9, L44, L45 and was absent in L20, L40 and L51, despite the fact that varieties with this gene were present in the pedigrees of each of these lines. The *Lr6Agi* was identified in the L7 and L20 lines with the participation of Favorit and Belyanka varieties, as well as in L40 and L45, in the pedigree of which the indicated varieties were absent.

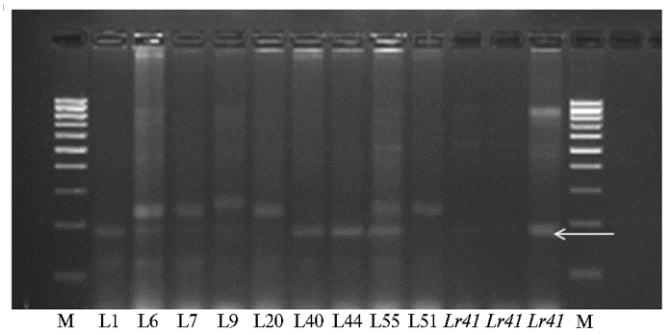

FIg. 3. PCR identification of *Lr39*(=*Lr41*) gene microsatellite marker GDM35 in introgression lines (L) **of spring bread wheat (ARISER):** M — molecular weight marker (DNA length marker 100 bp, Diaem, Russia), *Lr41* — positive control (line KS-90-WGRC-10). Arrow indicates 180 bp PCR product.

When analyzing plants individually, we revealed *Lr6Agi* gene segregation for L40 line and stable inheritance for L45 line. So additional cytogenetic analyzes or molecular PLUG markers-based analysis (PCR-based landmark unique gene) [51, 52] are necessary to finally confirm the presence of this gene. Additional *Lr26* gene was identified in L7, L9, and L44 lines, the probable source of which was the Weaver line of the synthetics pedigree.

All lines with the synthetic amphidiploid in pedigrees were highly resistant. For L7 *Lr10* + *Lr26* + *Lr6Agi*), L9 (*Lr19* + *Lr26*), L20 (*Lr10* + *Lr6Agi*), L40 (*Lr10* + *Lr39* + *Lr6Agi*), L44 (*Lr19* + *Lr26* + *Lr39*), L51 (*Lr6Agi*), and L45 (*Lr10* + *Lr19* + *Lr39* + *Lr6Agi*) this is consistent with the data on the genetic control. In L6, we can assume the presence of another gene, since upon inoculation with clone No. 2 virulent to *Lr19*, its resistance was observed (see Table 1).

Group IV. A susceptible sample of the hexaploid *T. petropavloskyi* and the resistant variety Voevoda participate in lines L21 and L3. Line L21 was characterized by susceptibility both of seedlings and adult plants, which indicates the absence of genetic material from the Voevoda variety. This is confirmed by molecular analysis. In L21, no markers of *Lr6Agi* gene were detected, but an ineffective *Lr10* gene was identified. The highly resistant L31 line carries the *Lr6Agi* gene which, probably, determines the L31 resistance.

In general, the *T. petropavloskyi* is characterized as highly susceptible to

fungal diseases [45]. However, this species is of interest for breeding as a donor of other economically important and biological traits. Cytological analysis will help to assess the presence of the genetic material from *T. petropavloskyi* in the lines of this group.

Group V. The lines of this group were produced with the participation of hexaploid species *T. kiharae* and *T. miguschovae*. *T. kiharae* is a homologue of *T. spelta* L., and *T. miguschovae* was created as a homologue of *T. aestivum*. These species are important for breeding common wheat as donors of high productivity [53]. The *T. kiharae* forms used to produce L17, L18, L22, L32, L33, L33, and L53 lines was resistant to brown rust and in preliminary studies showed one dominant gene for resistance [12].

In all lines of this group, we detected *Lr19* gene individually (L17, L32) or in combination with other genes, the *Lr19* + *Lr28* for L18; *Lr10* + *Lr19* for L22; *Lr3* + *Lr19* + *Lr28* for L33; *Lr3* + *Lr19* for L53. For lines L17, L22 and L32, these results are confirmed by a phytopathological test (susceptibility to clone No. 2). According to the genealogy of the lines L17, L18, L33, L53, the source of *Lr19* could be varieties Dobrynya and L503, while L22 and L32 were produced on the basis of varieties Saratovskaya 68 and Saratovskaya 70, in which this gene is absent.

Lines L18 and L33 had an SCS421 marker associated with *Lr28* [22]. As shown above, this marker is not strictly specific for the Lr28 gene translocation from *Ae. speltoides* and is detected in samples obtained with the participation of *T. timopheevii* [48]. Hypothetically, it can be assumed that the detection of this marker in L18 and L33 indicates the presence of the *T. kiharae* genetic material. This is confirmed by the high resistance to the disease in the field and lab tests. Since among the known *Lr* genes there are no transmitted from this species [42], we can assume that they are new and not identical to the known effective ones (*LrTkh*).

In lines L56, L57, L58 obtained with the participation of *T. miguschovae*, the resistance type varied upon infection of seedlings with populations and clones. Adult plants of L56 line were highly resistant, the other two lines showed resistance segregation. Molecular marker detected ineffective *Lr3* and *Lr1* genes and the partial resistance gene *Lr34* in L56 and L57. However, these genes were not found in the L58 line of similar origin.

An analysis of 42 promising wheat lines showed high genetic diversity in brown rust resistance. Among them there were carriers of known *Lr* genes not previously used in spring bread wheat breeding in Russia (*Lr29* in L4), and the carriers of presumably new *Lr* genes from *T. durum* (L8, L39 *Lr19* + *LrTdur*, L25, L19, L11 — *Lr10* + *Lr19* + *LrTdur*), *T. persicum* (L38 — *Lr19* + *LrTpers*), *T. timopheevii* (L49 — *Lr10* + *LrTtim*), *Ae. tauschii* (L6 — *Lr19* + *LrAtau*), *T. kiharae* (L33 — *Lr3* + *Lr19* + *LrTkh*). Moreover, we have identified carriers of effective combinations of *Lr* genes: *Lr19* + *Lr26* (L10, L13, L46, L24, L48, L5, L9), *Lr19* + *Lr37* (L42), *Lr10* + *Lr19* + *Lr26* (L2, L28, L29), *Lr19* + *Lr26* + *Lr39* (L44), *Lr19* + *Lr37* + *Lr6Agi* (L3), *Lr19* + *Lr6Agi* (L4), *Lr10* + *Lr26* + *Lr6Agi* (L7), *Lr10* + *Lr39* + *Lr6Agi* (L40), as well as *Lr10* + *Lr19* + *Lr39* + *Lr6Agi* (L45) and *Lr1* + *Lr3* + *Lr34* (L56, L57).

Most of the identified alien *Lr* genes are in linkage groups with effective disease resistance genes. In one translocation with *Lr19*, there is a highly efficient stem rust resistance gene *Sr25*. The rye translocation 1BL.1RS, along with the *Lr26* gene, contains genes for resistance to powdery mildew (*Pm8*), stem (*Sr31*) and yellow (*Yr9*) rust, and translocation with *Lr37* gene of *Ae. ventricosa* contains genes for resistance to stem (*Sr38*) and yellow (*Yr17*) rust, cercosporellose root rot (*Pch2*), and cereal cyst forming nematode (*Cre5*). The *Lr34* gene is

closely linked to the genes of resistance to powdery mildew (*Pm38*), stem rust (*Sr57*) and yellow rust (*Yr18*) [12, 42]. Lines with these translocations will have group resistance to several diseases.

Virulence of the pathogen. In 2017 and 2018, along with immunological studies of introgression lines of spring wheat, we monitored the virulence of the Saratov population of *P. triticina*. Infectious material was collected from susceptible varieties growing in the general crop with the studied set of lines. In both years, when the tester *Lr*-lines were inoculated with the combined population of *P. triticina*, the genes *Lr9*, *Lr24*, *Lr28*, *Lr29*, *Lr39*(= *Lr41*), *Lr42*, *Lr45*, *Lr47*, *Lr50*, *Lr51*, *Lr53*, *Lr6Agi* were high effective (score 0 and 0;). Lines with *Lr28*, *Lr29*, *Lr39*, *Lr51*, *Lr6Agi* were also characterized by high field resistance. The entire set of these genes may be of interest for breeding in the Volga Region and increasing genetic diversity of cultivated wheat varieties. The Thatcher lines with the *Lr44*, *Lr57* showed a moderate resistance of 2 to 2 ++. All other lines showed susceptibility with different intensities of the lesion.

In 2017 and 2018, 45 and 35 *P. triticina* monopustular isolates, respectively, were tested with 20 isogenic *Lr* lines. Isolates virulent to Tc*Lr19* line had a moderate frequency (16% in 2017 and 20% in 2018). The pathogen virulence rate for the Tc*Lr26* line was high (80% in 2017 and 77% 2018). All isolates virulent to *Lr19* carriers were avirulent to *Lr26*. Probably, this gene combination is "forbidden" for the pathogen. The confirmation could be the results of immunological studies and high resistance of the introgression lines L10, L13, L28, L46 and others carrying the *Lr19* + *Lr26* combination. A significant variation in the frequencies of the pathogen over the years was observed on lines with genes *Lr2a*, *Lr2b*, *Lr2c*, *Lr15* (20% in 2017 and 100% in 2018). Frequencies of the pathogen virulence to lines with *Lr1*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, and *Lr30* were consistently high in both years (100%). This explains the high damage to seedlings and adult plants of the varieties and line L10 with *Lr10* gene. However, the above *Lr* genes in combination with the adult resistance gene *Lr34* may have an additive effect on increasing field resistance. Such facts are described [54] and are noted in our study for lines L56 and L57. In 2017, the studied Saratov population (ARISER) was represented by three pathogen virulence phenotypes (races), the MHTKH, TGTTT, and THTTR, and in 2018 by two phenotypes, the TGTTT and THTTR.

The THTTR phenotype is widely distributed throughout Russia and is detected almost annually. All resistant lines in our study were immune to this phenotype. The TGTTT phenotype is most characteristic of the Volga populations, though also noted in other Russian regions [55]. Its unequal representation in the Saratov population by years can explain the variability in the damage to varieties and lines with *Lr19* gene.

Thus, we have characterized the genetic control of resistance to brown rust (*Puccinia triticina* Erikss.) in a new promising breeding material that combines resistance to leaf rust with adaptability to adverse environmental factors, productivity and grain quality. Its distinctive feature is the widespread use of leaf rust resistance genes from related species. Lines with resistance genes effective in the Lower Volga Region (*Lr29*), which are little used in breeding in Russia, were determined. Lines with effective combinations of known *Lr* genes and with combinations of known *Lr* genes with presumably new alien genes have been identified. Alien genes have been transferred from durum wheat varieties, *Triticum persicum*, *T. timopheevii* and *T. kiharae*, i.e., from both primary and secondary common wheat pool. The use of effective combinations of *Lr19*, *Lr26*, *Lr34*, *Lr37* genes linked to effective genes for resistance to other diseases will determine the resistance of new lines to a complex of diseases, which increases the

value of such combinations. The information we obtain on the composition of the brown rust pathogen population in the Saratov Region and its changes during 2017-2018 will be key for planning and conducting work on advanced selection for brown rust resistance.

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