

Leaf rust resistance genes identification in the spring bread wheat breeding material of the Agricultural Research Institute for South-East Regions of Russia

*Elena Gulyaeva¹ · Sergei Sibikeev² · Alexander Druzhin² · Ekaterina Shaydayuk²

¹All-Russian Institute of Plant Protection, St. Petersburg, Russia

²Agricultural Research Institute for South-East Regions, Saratov, Russia

Summary: Leaf rust caused by *Puccinia triticina* is a common bread wheat disease in the Volga Region of Russia and breeding for this disease resistance is a priority for the Agricultural Research Institute for South-East Regions wheat program. Knowledge of the effective resistance genes present in the germplasm is relevant when selecting for effective and more durable resistance. *P. triticina* races with virulence to *Lr9*, *Lr19*, *Lr26* and with other different virulence combinations and molecular markers of *Lr* genes were used to determine which seedling resistance genes might be present in the 68 bread wheat lines and cultivars. Studies have shown that the effective protection against leaf rust widespread in the Volga Region spring bread wheat cultivars is controlled by *Lr6.Ag* and *Lr6.Ag+Lr19* genes. In addition, cultivars carry *Lr10*, *Lr19*, *Lr10+Lr26* genes. It was found that in the studied set of lines the leaf rust resistance is determined by the following *Lr*-genes and its combinations: 9, 10, 19, 26, 34, 37, 41, Satu, 6.Ag. Moreover, usage frequency of *Lr19* is 89.5%, *Lr10* – 40.4%, *Lr26* – 31.6%, *Lr6.Ag* – 21%, *Lr28* – 3.5%, *Lr41* – 3.5%, *Lr9* – 1.8%, *Lr34* – 1.8%, *Lr37* – 1.8%, *LrSatu* – 1.8%. The frequency of two *Lr*-genes combinations is 45.7%, three – 21% and four *Lr*-genes – 5.3%. Mainly are used such *Lr*-genes combinations as: *Lr19+Lr26* and *Lr10+Lr19+Lr26* – 22.8%; *Lr19+Lr6.Ag* – 7%. The four *Lr*-genes combinations has been included *Lr10+Lr26+Lr28+Lr6.Ag* – 1.8%, *Lr1+Lr10+Lr26+Lr6.Ag* – 1.8% and *Lr10+Lr19+Lr28+Lr6.Ag* – 1.8%. In addition, the effective *Lr19* with non-identified *Lr*-genes from cultivar Saratovskaya 57 (L164) and *A. elongatum* (CI-7-57) combinations has been identified.

Key words: bread wheat, leaf rust, *Lr*-genes, molecular markers, resistance

Introduction

The best approach to control diseases such as leaf rust is genetic resistance based on effective resistance genes (Spanic et al., 2015). Unfortunately, many cultivars of winter and spring wheat do not contain effective race-specific resistance, and the breeding for non-racial specific resistance has not received sufficient development. Thus, due to its economic importance, resistance to leaf rust is a priority in the Agricultural

Research Institute for South-East Regions (ARISER) breeding program. Because, currently, the bread wheat intraspecific genetic variability is unable to provide a sufficient protection degree against pathogens, the most promising in this regard are related wheat species, representatives of the genera *Aegilops*, *Secale*, *Agropyron*. In ARISER Laboratory of Genetic and Cytology during the period 1990–2017, the leaf rust resistant germplasm from such species as *T. durum* Desf., *A. elongatum*, *A. intermedium* Host, *A. speltoides* Tausch, *A. ventricosa* Tausch, *Secale cereale* L., triticale cv. Satu and also synthetics of CIMMYT breeding were used. As a result, set of introgressive lines resistant to leaf and stem rust were obtained. It should be noted that the main direction was the several *Lr*-genes in one genotype integrating to prolong the resistance effectiveness against the pathogen. Breeding and selection for leaf rust resistance in wheat cultivars can be facilitated by extensive genetic analyses using molecular markers that are linked or resistance specific (Kolmer et al., 2013).

Corresponding author:
eigulyaeva@rambler.ru

Acknowledgement:
Supported by Russian Foundation for Basic Researches, grant №18-016-00170a

Cite this article:
Gulyaeva E., Sibikeev S., Druzhin A., Shaydayuk E. (2019). Leaf rust resistance genes identification in the spring bread wheat breeding material of the Agricultural Research Institute for South-East Regions of Russia. *Ratar. Povrt.*, 56 (2), 34-40.

Also gene postulations are possible because of gene-for-gene specificity, where the infection types produced by pathogen isolates on wheat cultivars under study are compared to infection types produced by the same isolates on the so-called differentials, often near isogenic lines, each carrying known single resistance gene (Pathan, Park 2007). But this method has a limitation because if the sample carries two *Lr*- genes it is not always possible to find the corresponding clone test. For example, in the Volga region *Lr19* and *Lr26* genes are inefficient in protecting wheat from leaf rust, despite their combination efficacy (*Lr19+Lr26*). Accordingly, the clones virulent to *Lr19* and *Lr26* are widely found in the pathogen population, but there are none virulent to both. In this case marker-assisted selection (MAS) combined with traditional breeding techniques has become a valuable tool in selection of individuals carrying genes, controlling the traits of interest, such as disease resistance.

The aim of this study was to determine the leaf rust resistance genes presence in 57 wheat introgression lines and 11 cultivars developed at the ARISER and to postulate the known genes presence based on infection type caused by well-characterized races of these pathogens and using molecular markers.

Materials and Methods

Sixty-eight spring bread wheat lines and cultivars (Table 1) developed at ARISER Russia and highly resistant to *P. tritici* in the field were tested at the seedling stage for infection types to postulate seedling leaf rust resistance genes. Ten seedlings of each line were grown in the pots with soil and were inoculated with urediniospores suspension of single *P. tritici* race when the first leaf had been fully expanded. Characteristics of using races to 27 isogenic lines are presented in Table 2. All these 5 isolates had susceptible infection type (3-4) to the Thatcher line with *Lr1*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr21* and *Lr30*, and resistant type to the Thatcher line with *Lr24*, *Lr28*, *Lr29*, *Lr41* and *Lr47*. The main variation among races included virulence to *TcLr9*, *TcLr19* and *TcLr26*.

Plants were kept in 100 % humidity chamber overnight and then maintained in the Versatile Environmental Test Chamber MLR-352H (SANYO Electric Co., Ltd., Japan) at 22° C. Infection types (IT) were classified on 0–4 scale during 10–12 days after inoculation on seedlings as described by E.B. Mains and H.S. Jackson (1926), where 0 – no visible uredia; 0; – hypersensitive flecks; 1 – small uredia with necrosis; 2 – from small to medium sized uredia with green islands surrounded by necrosis or chlorosis; 3 – medium sized uredia with or without chlorosis; 4 – large uredia without chlorosis; X – heterogeneous, similarly distributed over the leaves. The plants with 0 to 2 infection types were classified as resistant, 3 to 4 and X infection types as susceptible.

Leaf rust resistance genes (*Lr*) in wheat cultivars and lines were also determined by molecular markers WR03 (*Lr1*), SCS5 (*Lr9*), F1.2245/*Lr106/r2* (*Lr10*), SCS265 (*Lr19*), STS638 (*Lr20*), *Sr24#12*, *Sr24#50* (*Lr24*), *scm9* (*Lr26*), SCS421 (*Lr28*), *Lr29F24* (*Lr29*), *csLV34* (*Lr34*), *Sr39=22* (*Lr35*), *Verntrip/LN2* (*Lr37*), GDM35 (*Lr41*), PS10 (*Lr47*) and S13-R16 (*Lr66*) (<http://maswheat.ucdavis.edu>; Gupta et al., 2005, 2006; Cherukuri et al., 2005; Qiu et al., 2007; Weng et al., 2007; Marais et al., 2010). For *Lr10*, *Lr26*, *Lr34* and *Lr37* genomic DNA was extracted from leaves of three plants belonging to each wheat line using Dorokhov and Kloke (1997). The PCR reactions were carried out as proposed previously (Gulyaeva et al., 2009). The amplified products were visible under UV light after electrophoresis on 1.5% agarose gels containing ethidium bromide.

Results

Resistance of 68 introgressive lines and cultivars to leaf rust isolates at seedling plant stages is shown in Table 1. Thirty five wheat samples had resistance (IT=0) to all races used for multi-pathotype tests. Accordingly, their resistance can be controlled by highly effective *Lr*-genes, or their effective combinations. Using molecular markers, the combination of *Lr19* and *Lr26* genes was determined for lines №1, №13, №32-33, №36 and №38. In lines №6, №19, №22, №34, №40-41, №45-46, along with these genes, *Lr10* was determined, and in line №20 – *Lr41*-gene. The presence of *Lr41*gene in line №20 is probably due to the presence of *A. squarosa* (= *A. tauschii* Coss.) in pedigree. In addition, a combination of *Lr19+Lr41* genes was determined in line №35. Effective *Lr9* and *Lr19* genes combination is revealed in the line №18.

Genes *Lr10* and *Lr19* were revealed in the high-resistant introgressive lines №26, №48, №55-56, and *Lr19* gene in the lines №3, №12, №15, №31 and №42. Their resistance to all used races predicts the additional genes presence beyond *Lr19* gene, since other lines carrying *Lr10+Lr19*, demonstrated a susceptible reaction (Table 1). In line №3, according to its pedigree, the additional gene may be *Lr25*. Genes from durum wheat L164 (Saratovskaya 57) in line №15 may be additional. In lines №42, №48 and №55 gene *Lr6Ag* originates from cultivars Belyanka, Favorit and lines Multi 6R, accordingly. Line №31 has a source of *Lr24* gene in its lineage, but this gene has not been identified. Probably, the resistance of this line can be provided by an additional unknown or just a new gene. Furthermore, additional or new resistance genes to leaf rust can be found in lines №26 and №56, in the first one originates from *A. elongatum* (CI-7-57 2n=70), and in the second one originates from the Australian *Sr35* gene source.

Lr1 and *Lr19* genes and *LrSp* gene originates from *A. speltoides* were revealed in line №10. The *LrSp* gene is

Table 1. Characteristic of bread wheat breeding lines and cultivars for resistance to leaf rust

№	Pedigree	Lr genes	Infection type to races				
			R1	R2	R3	R4	R5
1	L2032*6/Curinda87	Lr19 Lr26	0	0	0;	0	0
2	Dobrunya*5/Tc Lr9	Lr10 Lr19	0	3	0	0	0
3	Dobrunya*4/Tc Lr25	Lr19	0	0;	0	0	0
4	Dobrunya*5//Milan/Prinia L653	Lr19	0	3-4	0	0	0
5	Dobrunya*5//Milan/Prinia L654	Lr19	0	3-4	0	0	0
6	L503 Lr19 Lr26	Lr10 Lr19 Lr26	0	0	0	0	0
7	Saratovskaya55/Ag. el *6// Saratovskaya 29	Lr19	0	3-4	0	0	0
8	S55/Ag.el*4//S29/3/ L1015 ?Ag ^e (3B)/?Ag ^e (3D)	Lr19	0	3-4	0	0	0
9	L505*2//L503*2/Cuckoo line L195	LrSp	0	0	0	0	0
10	L505//L503//L583/ Cuckoo line //L505 L200	Lr1 Lr19LrSp	0	0	0	0	0
11	S55*3/T. dic-s//L2032	Lr10	0	0	0	0	0
12	Dobrunya /Prohorovka//Dobrunya	Lr19	0	0	0	0	0
13	L2032*5/Seri82	Lr19 Lr26	0	0	0	0	0
14	L505*2//Croc/Ae.squar(224)//Yaco	Lr19Lr34	0	3-4	0	0	0
15	Lud//C55*2/T.dic-s//Saratovskyzolotistaya./L164//S55	Lr19	0	0;2	0	0	0
16	S55/Ag. el*6// S29	Lr19	0	3-4	0	0	0
17	Dobrunya*4/Nik	Lr19	0	3	0	0	0
18	Dobrunya*4/3/Croc/Ae.squar(205)//Weaver	Lr9 Lr19	0	0	0	0	0
19	L505 /3/Croc/Ae.squar(205)//Weaver/4/L505/5/C68	Lr10 Lr19 Lr26	0	0	0	0	0
20	L505 /3/Croc/Ae.squar(205)//Weaver/4/L505/5/L505	Lr19 Lr26 Lr41	0	0	0	0	0
21	Belyanka/3/Croc/Ae.squar(205)//Weaver/4/ Belyanka	Lr10 Lr26	0	0	0	0	0
22	Croc/Ae.squar(205)//Weaver/3/L505/4/ Belyanka	Lr10 Lr19 Lr26	0	0;	0	0	0
23	Croc/Ae.squar(205)//Weaver/3/L505/4/ DobrunyaLr25	Lr19	0	3	0	0-1	0
24	L12(Dobrunya Lr24) /S68//S68	Lr10 Lr19	0	2+	0	0	0
25	S55/Ag. el *5// S29 (Ag ^e -7D)/3/S68	Lr10 Lr19	0	3-4	0	0	0
26	C55/Ag. el*5// C29 (Age-7D)/3/C68	Lr10 Lr19	0	0	0	0	0
27	Belyanka/3/Altar84/ Ae.squar(224)//Pgo/4/C68 (L481/16)	Lr10 Lr19	0	3-4	0	0	0
28	L505*2/Prohorovka// Belyanka (L496/16)	Lr10 Lr26 Lr28?	0	0	0	0	0
29	S55*3/T. dic-s//L2032 (L501/16)	Lr1 Lr10Lr26	0	0	0	0	0
30	Dobrunya//6R/Agis181 (L426/16)	Lr10	0	0	0	0	0
31	Dobrunya/Zolotayavolna// Dobrunya Lr24/3/Dobrunya	Lr19	0	0	0	0	0
32	L505/C42/4/L505*3//Proh//L505/3/S70/4/ DobrunyaLr24	Lr19 Lr26	0	0;	0	0	0
33	L505/L164/4/L503//TTrap#1/Bow/3/L503/5/L505/6/C68	Lr19 Lr26	0	0	0	0	0
34	YV2/L505//L503 Lr26/3/L505/4/S68	Lr10 Lr19 Lr26	0	0	0	0	0
35	Croc/Ae.squar(205)//Weaver/3/L505/4/ DobrunyaLr25	Lr19 Lr41	0	0	0	0	0
36	Dobrunya*5/TcLr9//L505//L503*3/TRAP≠BOW/Proh/S55	Lr19 Lr26	0	0	0	0	0
37	L528//C55*4/T.dic-s	Lr19	0	3	0	0	0
38	Saratovsk zolotistaya/T.dic-s//C58/3/ *2 Belyanka/4/Voevoda	Lr19 Lr26	0	0	0	0	0
39	L503Lr26/ Ottan(R11,R12)//Revansh	Lr10 Lr19	0	3-4	0	0	0
40	L18(L503 Lr26)/C68//Revansh	Lr10 Lr19 Lr26	0	0	0	0	0
41	Tulaikovskaya 10// Agis181/C29+Agis181/S58	Lr10 Lr19 Lr26	0	0	0	0	0
42	Dobrunya Lr25/Belyanka//L505	Lr19	0	0;	0	0	0
43	Thatcher Lr37*4/L503	Lr10 Lr19	0	3-4	0	0	0
44	Lebedushka	Lr19 LrAgj	0	0;	0	0	0
45	L503Lr26/ Ottan(R11,R12)//Revansh	Lr10 Lr19 Lr26	0	0	0	0	0
46	YV2/L505//L503 Lr26/3/L505/4/C68	Lr10 Lr19 Lr26	0	0	0	0	0
47	Favorit/ W3534	Lr19	0	3	0	0	0
48	Milan/Prinia//*4 Dobrunya/3/Favorit	Lr10 Lr19	0	0	0	0	0
49	S55/Ag. el *6// S29	Lr10 Lr19	0	3-4	0	0	0
50	Prohorovka*2/L164*2//L164	Lr10 Lr19	0	0-1	0	0	0
51	Celinnaya20/ Dbr// Dbr/3/ DbrLr9/4/Milan/Prinia*4//Dbr	Lr37 Lr19	0	3,0	0	0	0
52	Croc/Ae.squar//Weaver/3/*4 L505/4/S73	Lr10 Lr19	0	3-4	0	0	0
53	S70*4/3/ Croc/Ae.squar(224)//Yaco	Lr19	0	3	0	0	0

54	Dobrunya*5//Milan/Prinia/3/Belyanka/4/S68	<i>Lr10 Lr19</i>	0	3-4	0	0	0
55	Prohorovka/MultiLr6R//S68/3/ Dobrunya	<i>Lr10 Lr19</i>	0	0;	0	0	0
56	L503/ Sr 35//L503/3/L503	<i>Lr10 Lr19</i>	0	0;	0	0	0
57	S74/T.dicoccum k7507*3//S73	<i>Lr10 Lr19</i>	0	3,0	0	0	0
58	Satu/S70//S74/3/S74	<i>Lr10</i>	0	0;	0	0	0
59	Saratovskaya 55 (S55)		3	3	3	3-4	3
60	Saratovskaya 68 (S68)	<i>Lr10</i>	3	3-4	3-4	3-4	3
61	Saratovskaya 70 (S70)		3	3-4	3-4	3-4	3
62	Saratovskaya 73(S73)	<i>Lr10</i>	3	3	2	2	3
63	Albidum 32	<i>Lr10</i>	3	3-4	3-4	3-4	3
64	Prohorovka	<i>Lr10 Lr26</i>	0	0	3	0	3
65	Yugo-Vostochnaya 2 (YV2)	<i>Lr10Lr26</i>	0	0	3	0	3
66	Dobrunya	<i>Lr19</i>	0	3	0	0	0
67	Favorit	<i>Lr6Agj</i>	0	0	0	0	0
68	Voevoda	<i>Lr6Agj</i>	0	0	0	0	0

Table 2. Characteristic of *Puccinia triticina* races used in multi pathotype tests

Races	Virulence to <i>Lr</i> genes	Avirulence to <i>Lr</i> genes
R1	1,2a,2b,2c,3a,3bg,3ka,9,10,11,14a,14b,15,16,17,18,20,21,30	19, 23,24,26,28,29,41,47
R2	1,2a,2b,2c,3a,3bg,3ka,10,11,14a,14b,15,16,17,18,19,20,21,30	9, 23,24,26,28,29,41,47
R3	1,2a,2b,2c,3a,3bg,3ka,10,11,14a,14b,15,16,17,18,20,21,26,30	9, 19,23,24,28,29,41
R4	1,2a,2b,2c,3a,3bg,3ka,10,11,14a,14b,15,16, 17,18,20,21, 30	9, 19,23,24,26,28,29,41,47
R5	1,2b,2c,3a,3bg,3ka,10,11,14a,14b, 16, 17,18,20,21,26,30	2a,9,15,19,23,24,28,29,41

also identified in line №9. This gene is transferred to these samples from the Cuckoo line, developed with *A. speltoides* participation.

The low-efficiency *Lr10* gene was detected in the highly resistant lines №11 and №58. In the line №29 the combination of inefficient genes *Lr1*, *Lr10* and *Lr26* was revealed, in line №21 – *Lr10* and *Lr26*. Undoubtedly, these genes can play a role in these lines resistance strengthening, but lines' №11 and №29 resistance is ensured the most likely by the presence of additional new or unidentified genes from *T. dicoccoides*, in line №58 – from triticale Satu (*LrSatu*), in the line №21 – from cultivar Belyanka (*Lr6Agj*), since the *Lr41* gene from *A. tauschii* hasn't been identified in this line.

The high resistance of cultivars Favorit (№67) and Voevoda (№68) is provided by the gene *Lr6Agj* (Sibikeev et al., 2017), an effective marker for which is not provided. Genes *Lr10*, *Lr26* and *Lr28* were specified in line №28 and genes *Lr10* and *Lr28* –in line №30. At the same time, the amplicon size in the Tc*Lr28* line was slightly lower than that of these two lines. It was shown that the SCS421 marker was not strictly specific for this gene (Serfling et al., 2011). *Lr28* gene was transferred to bread wheat from *Ae. speltoides* (McIntosh et al., 1995). Donors *A. speltoides* in pedigree of these lines are absent so this marker presence in lines №28 and №30 may be a false positive result indication. In line №28, according to its pedigree, it can be

expected *Lr6Agj* gene presence from cultivar Belyanka, in line №30 from the Agis181 line having the genetic material from *A. intermedium* (Badayeva E.D., unpublished data).

Along with highly resistant lines and cultivars, 24 lines were identified in the studied collection, which were resistant to all races used, except R2 race which was virulent to Tc*Lr19* (Table 1). According to the multi-pathogen test, *Lr19* gene presence can be assumed in these samples. These lines also performed the yellow color of the flour, i.e. a feature linked to *Lr19* gene presence. There were cultivars Dobrunya, L503, L505, carrying this gene in the most lines pedigree. Using the marker SCS265, *Lr19* gene presence was confirmed in the introgressive lines №2, №4-5, №7-8, №14, №16-17, №23, №24, №25, №27, №37, №39, №43, №47, №49-50, №52-54, №57 and cultivar Dobrunya (№66). In most of the studied lines, *Lr19* gene with other ineffective *Lr*-genes combination was determined, and these actions were not observed in the phytopathological test seedling stage. Their presence in the lines genotypes practically did not affect the reaction type under test clone R2 inoculation in the seedling phase. Partial resistance *Lr34* gene was identified in line №14 which effect appeared in the adult wheat plants phase. The inefficient *Lr10* gene, which is widely distributed in ARISER cultivars, has been identified in the lines №2, №24-25, №27, №39,

№47, №49-50, №52, №54 and №57. All these lines demonstrated IT= 3 or 3-4 while infected with test clone R2 virulent to *Lr19* except line №24, which IT = 2+. This may point at the additional *Lr*-genes presence in this line. Adult plant resistance *Lr37* gene was identified in line №51, probably transferred from cultivars Milan or Prinia. However, resistance cleavage in this line was observed after inoculation by isolates virulent to *Lr19* (R2).

Cultivars Prohorovka and Yugo-Vostochnaya 2 were susceptible to clones virulent to *Lr26*, indicating that they possessed this gene. This fact was also confirmed using scm9 marker. Along with this one, *Lr10* gene was identified in these cultivars. Cultivars Saratovskaya 55, Saratovskaya 68, Saratovskaya 70 and Albidum 32 were susceptible by all used test isolates, and the cultivar Saratovskaya 73 performed a variation in resistance. Ineffective *Lr10* gene was revealed in Saratovskaya 68 and Saratovskaya 73 cultivars, which had a wide representation in ARISER selection cultivars.

Studies have shown the effective protection against leaf rust having the widespread in Volga Region of Russia spring bread wheat cultivars is controlled only by *Lr6Ag* and *Lr6Ag+Lr19* genes. In addition, cultivars carry *Lr*-genes – *Lr10*, *Lr19*, *Lr10+Lr26*. It was found that in the studied ARISER breeding set of lines the leaf rust resistance is determined by the combinations following *Lr*-genes: 9, 10, 19, 26, 34, 37, 41, Satu, *6Ag*.

Discussion

In the Volga region *P. triticina* is the most severe parasite on both winter and spring bread wheat. The leaf rust epiphytotics are observed on average once every three to four years. In such years, the wheat harvest is reduced up to 20–30% or more, protein and gluten content in the grain is significantly reduced as well. The analysis of the pathogen epiphytotic chronology shows that in the Volga region the damage from leaf rust has become stronger in recent times than in the first half of the 20th century. The following factors are the main to favor the epiphytotics development. First of all, these are structural changes in wheat acreage. Until 1960s wheat in the Volga region was represented only by spring bread and spring durum wheat, the conditions for maintaining the local *P. triticina* inoculums were unfavorably time-limited (April-August). Currently, the share of spring durum wheat in total wheat crops is less than 10%, spring bread wheat is no more than 25%, but winter bread wheat is more than 65%. Thus, at present moment from spring until late autumn the leaf rust inoculum propagated on live plants both winter and spring wheat. In addition, in the Volga region, there is no wide diversity of cultivars with various resistance genes to leaf rust. For example, in 1960s and 1970 winter bread wheat cultivars Mironovskaya 808 and Bezostaya 1 entered the fields,

which carried gene *Lr3*, and then there were spring bread wheat cultivars, which also contained a single from *Lr*-genes – Saratovskaya 46 (*Lr14*), Ershovskaya 32 (*Lr23*). In the early 1990s through Volga and the Urals regions there was a rapid widespread of L503, L505 and Samsar cultivars containing the *Lr19* gene from *A. elongatum*. After reaching these cultivars the "critical" plantings area (about 100 thousand hectares), the first pustules of *P. triticina* were found in fields, in Saratov and Orenburg regions simultaneously (Sibikeev et al., 1996). *Lr19*-gene usage quickly changed *P. triticina* population structure. To the end of 20th century in Volga region among the studied 28 *Lr*-genes already 22 were overcome by pathogen, only *Lr9* and *Lr24* showed high efficiency (Sibikeev et al., 2007). Wheat rust resistance genetics information in Volga regions is widely available (Gultayeva et al., 2012; Sibikeev et al., 2017). Based on the available information and present research it could be concluded that ARISER wheat leaf rust resistance is based on *Lr19*, *Lr26*, *Lr6Ag* and *Lr10*. Cultivars L503, L505, Dobrynya have the genes *Lr10+Lr19*, Belyanka, Favorite, Voevoda – *LrAg*, Lebedushka – *Lr6Ag+Lr19* (Sibikeev et al., 2017). Leaf rust epiphytotics in 2016–2017 vegetation period in Saratov region showed only Belyanka, Favorite, Voevoda and Lebedushka with genes *LrAg*, and *Lr6Ag+Lr19* having high-efficiency resistance to the pathogen. Breeding and genetic programs for production bread wheat cultivars resistant to leaf rust are impossible without pathogen and its virulent properties study. Studies have shown that many races are generally present in the *P. triticina* population in Low Volga region and their relative frequencies may change significantly from one year to another (Ivanova et al., 2011). Races with new virulence combinations often overcome cultivars resistance a few years after release. In connection with the above, it is necessary to have and produce a sufficient number of effective genes of resistance to leaf rust and their combinations to provide a diversity of cultivars for them. Our study showed the following diversity and frequency of *Lr*-genes usage: *Lr19* – 89.5%, *Lr10* – 40.4%, *Lr26* – 31.6%, *Lr6Ag* – 21%, *Lr28?* – 3.5%, *Lr41* – 3.5%, *Lr9* – 1.8%, *Lr34* – 1.8%, *Lr37* – 1.8%, *LrSatu* – 1.8%. The frequency of two *Lr*-genes combinations is 45.7%, three – 21% and four *Lr*-genes – 5.3%. Mainly used are such *Lr*-genes combinations as: *Lr19+Lr26* and *Lr10+Lr19+Lr26* – 22.8%; *Lr19+Lr6Ag* – 7%. Into the four *Lr*-genes combinations has been included *Lr10+Lr26+Lr28?+Lr6Ag* – 1.8%, *Lr1+Lr10+Lr26+Lr6Ag* – 1.8% and *Lr10+Lr19+Lr28?+Lr6Ag* – 1.8%. In addition, the effective combination of *Lr19* with non-identified *Lr*-genes from durum wheat cultivar Saratovskaya 57 (L164) and *A. elongatum* (2n=70 CI-7-57) combinations has been detected. The different countries planting bread wheat have different situation for the diversity of genes for resistance to leaf rust in cultivars and primary breeding material. A study in China in 2015 of 84 bread

wheat cultivars by test the virulence of 12 races of *P. triticina* and 8 molecular markers of *Lr* genes showed that twelve *Lr* genes, including *Lr1*, *Lr3*, (*Lr3bg*), (*Lr3ka*), *Lr11*, *Lr13*, *Lr14a*, *Lr16*, *Lr26*, *Lr27*, *Lr30* and *Lr31* were postulated to be present either singly or in combinations. *Lr3* and *Lr26* were detected most often in the tested cultivars, with frequencies of 51.2 and 38.1%, respectively. No wheat *Lr* genes were detected in 16 cultivars, and 4 cultivars may carry unknown *Lr* genes (Xiao-li Ren et al., 2015). In China in 2018, 35 cultivars were tested by 16 Pt-rases and 15 *Lr* genes molecular markers. Seedlings of 20 tested wheat cultivars showed high resistance phenotype to at least one Pt race. Only three *Lr* genes (*Lr1*, *Lr10* and *Lr26*) were identified in some of the tested cultivars. Five cultivars including Liangxing 66, Hemai 9735, Luyuan 301, Jimai 17, and Taishan 027 showed typical APR reaction in the field. These results indicate that the diversity of known *Lr* genes in the tested wheat cultivars is relative low (Li J et al., 2018). In the Egypt in 2014 the similar study showed ten genes, *Lr13*, *Lr19*, *Lr24*, *Lr26*, *Lr34*, *Lr35*, *Lr36*, *Lr37*, *Lr39*, and *Lr46*, were detected in fifteen wheat cultivars. The most frequently occurring genes in fifteen Egyptian wheat cultivars were *Lr13*, *Lr24*, *Lr34*, and *Lr36* identified in all the cultivars used, followed by *Lr26* and *Lr35* (93%), *Lr39* (66%), *Lr37* (53%), and *Lr46* (26.6%) of the cultivars, and finally *Lr19* was present in 33.3% of cultivars. It is concluded that there was a good variation in *Lr* genes carried by wheat cultivars commercially grown in Egypt (Imbaby et al., 2014).

Conclusion

The studied breeding lines will be used in breeding programs in Volga region of Russia to expand the genetic diversity of wheat varieties by using alien genes.

References

- Cherukuri, D.P., Gupta, S.K., Charpe, A., Koul S., Prabhu, K.W., Singh, R.B., Haq, Q.M.R. (2005). Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. *Euphytica*, 143, 19-26.
- Dorokhov, D.B. and Kloke, E. (1997). Rapid and economical technology of RAPD analysis of plant genomes. *Mol. Genet.*, 33(4), 358-365.
- Gulyaeva, E. I., Kanyuka, I. A., Alpatova, N. V., Baranova, O. A., Dmitriev, A. P., Pavlyushin, V. A. (2009). Molecular approaches in identifying leaf rust resistance genes in Russian wheat varieties. *Russian Agricultural Sciences*, 35, 316-319.
- Gulyaeva, E.I., Ivanova, O.V., Markelova, T.S., Sibikeev, S.N. (2012). Identification of leaf rusts resistance genes at the introgression bread wheat cultivars and lines produced in the Agricultural Research Institute for South-East Regions of Russia by phytopathologic test and molecular markers. *Plant Protection News*, 1, 38-44. (in Russian)
- Gupta, S.K., Charpe, A., Prabhu, K.W., Haque O.M.R. (2006). Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. *Theor. Appl. Genet.*, 113, 1027-1036.
- Gupta, S.K., Charpe, A., Koul, S Prabhu, K.V., Haq, Q.M. (2005). Development and validation of molecular markers linked to an *Aegilops umbellulata*-derived leaf rust- resistance gene, *Lr9*, for marker-assisted selection in bread wheat. *Genome*, 48, 823-830.
- Imbaby I.A., Mahmoud, M.A., M. Hassan, M. E., Abd-El-Aziz, A. R. M. (2014). Identification of leaf rust resistance genes in selected Egyptian wheat cultivars by molecular markers. *The Scientific World Journal* ID 574285, 7 p.
- Ivanova, O.V., Markelova, T.S. (2011). The dynamics of structure population of *Puccinia recondita* f.sp.tritici Rob. et Desm in the Volga region. *Protection and Quarantine of Plants*, 9, 20-21. (in Russian)
- Kolmer, J.A., Mert, Z., Akan, K., Demir, L., Ünşal, R.S., Ermet, C., Keser, M., Akin, B., Morgounov, A. (2013). Virulence of *Puccinia triticina* in Turkey and leaf rust resistance in Turkish wheat cultivars. *Eur. J. Plant Pathol.*, 135, 703-716.
- Li, J., Shi, L., Wang, X., Zhang, N., Wei, X., Zhang, L., Yang, W., Liu, D. (2018). Leaf rust resistance of 35 wheat cultivars (lines). *J. Plant Pathol. Microbiol.*, 9, 429.
- Mains, E.B., Jackson, H.S. (1926). Physiologic specialization in the leaf rust of wheat; *Puccinia triticina* Erikss. *Phytopathology*, 16:89-120.
- Marais, G.F., Bekker, T.A., Eksteen A., Fetch, T., Maccallum, B., Marais, A.S. (2010). Attempts to remove gametocidal genes co-transferred to common wheat with rust resistance from *Aegilops speltoides*. *Euphytica*, 171, 71-85.
- McIntosh, R.A., Wellings, C.R., Park, R.F. (1995). *Wheat rusts. An atlas of resistance genes*. CSIRO Australia, Kluwer Acad. Publ., Dordrecht, the Netherlands.
- Pathan, A.K., Park R.F. (2007). Evaluation of seedling and adult plant resistance to stem rust in European wheat cultivars. *Euphytica*, 155, 87-105.
- Qiu, J.W., Schürch, A.C., Yahiaoui, N., Dong, L.L., Fan, H.J., Zhang, Z.J., Keller, B., Ling, H.Q. (2007). Physical mapping and identification of a candidate for the leaf rust resistance gene *Lr1* of wheat. *Theor Appl Genet.*, 115,159-168.
- Ren, X., Liu, T., Liu, B., Gao, L., Chen, W. 2015. Postulation of seedling leaf rust resistance genes in 84 Chinese winter wheat cultivars. *Journal of Integrative Agriculture*, 14, 1992-2001.
- Serfling, A., Krämer, I., Lind, V., Schliephake, E. (2011). Diagnostic value of molecular markers for *Lr* genes and characterization of leaf rust resistance of German winter wheat cultivars with regard to the stability of vertical resistance. *European Journal of Plant Pathology*, 130, 559-575.
- Sibikeev, S.N., Krupnov, V.A., Voronina, S.A., Elesin, V.A. (1996). First report of leaf rust pathotypes virulent to highly effective *Lr* genes transferred from *Agropyron* species to bread wheat. *Plant Breeding*, 115, 276-278.
- Sibikeev, S.N., Krupnov, V. A. (2007). Evolution of leaf rust and protecting of wheat from it in the Volga region. *Bulletin of Saratov State Agrarian University after name N. I. Vavilova*, pp.92-94.
- Sibikeev, S.N., Druzhin, A.E., Badaeva, E.D., Shishkina, A.A., Dragovich, A.Y., Gulyaeva, E.I., Kroupin, P.Y., Karlov, G.I., Khuat, T.M., Divashuk, M.G. (2017). Comparative analysis of *Agropyron intermedium* (Host) Beauv 6Agi and 6Agi2 chromosomes in bread wheat cultivars and lines with wheat-wheatgrass substitutions. *Russian Journal of Genetics*, 53, 314-324.
- Spanic, V., Rouse, M.N., Kolmer, J.A., Anderson, J.A. (2015). Leaf and stem seedling rust resistance in wheat cultivars grown in Croatia. *Euphytica*, 203, 437-448.
- Weng, Y., Azhaguvel, P., Devkota, R.N., Rudd, J.C. (2007). PCR based markers for detection of different sources of 1AL.1RS and 1BL.1RS wheat-rye translocations in wheat background. *Plant Breed.*, 126, 482-486.
- Xiao-li, R., Tai-guo, L., Bo, L., Li, G., Wan-quan, C. (2015). Postulation of seedling leaf rust resistance genes in 84 Chinese winter wheat cultivars. *Journal of Integrative Agriculture* 14(10), 1992-2001.

Identifikacija gena za otpornost na lisnu rđu u jarom oplemenjivačkom materijalu hlebne pšenice u Poljoprivrednom istraživačkom institutu za jugoistočne oblasti u Rusiji

Elena Gulyaeva · Sergei Sibikeev · Alexander Druzhin · Ekaterina Shaydayuk

Sažetak: Lisna rđa hlebne pšenice koju prouzrokuje *Puccinia triticina* je često oboljenje u oblasti Volge u Rusiji, a oplemenjivanje na otpornost na ovog patogena je prioritetni zadatak programa pšenice u Poljoprivrednom istraživačkom institutu za jugoistočne oblasti. Poznavanje efektivnih gena za otpornost koje su prisutne u germplazmi je važno pri oplemenjivanju na efektivnu i dugotrajniju otpornost. Korišćene su rase *P. triticina* sa virulentnošću na *Lr9*, *Lr19*, *Lr26* i sa drugim kombinacijama različitih virulentnosti i molekularni markeri *Lr* gena u cilju određivanja prisutnosti gena za otpornost u 68 linija i sorti hlebne pšenice. Rezultati pokazuju da se efikasna zaštita od lisne rđe široko rasprostranjena u jarim sortama hlebne pšenice u oblasti Volge kontroliše genima *Lr6Ag* i *Lr6Ag+Lr19*. Pored toga, sorte nose i gene *Lr10*, *Lr19*, *Lr10+Lr26*. U analiziranoj grupi linija, otpornost na lisnu rđu je određena sledećim *Lr*-genima i njihovim kombinacijama: 9, 10, 19, 26, 34, 37, 41, Satu, *6Ag*. Identifikovan je efikasan *Lr19* sa neidentifikovanim *Lr*-genima iz sorte Saratovskaya 57 (L164) i *A. elongatum* (CI-7-57) kombinacijama.

Cljučne reči: hlebna pšenica, lisna rđa, *Lr*-geni, molekularni markeri, otpornost

Received: 25 February 2019, Accepted: 12 April 2019

