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IDENTIFICATION OF INTROGRESSIVE WINTER WHEAT LINES WITH WILD RELATIVES PARTICIPATION BY RUST RESISTANCE GENES

Abstract. The genetic basis of winter wheat synthetic lines for leaf rust resistance was characterized by using molecular approaches. Molecular screening showed the presence of the Lr10 resistance gene for 24 lines (as Bezostaya 1 cvs and hybrids with it), for 4 samples-Lr13 (Zhetysu cvs and lines with participation), for 3 samples *Lr16* (Karlygash cvs and lines with its participation), in 8 samples - *Lr39* (*Lr41*) with *Ae.cylindrica* in the pedigree in combination with becrossing of cvs Erythrospermum 350 and cvs Steklovidnaya 24 and for 1 sample - *Lr62*, respectively. For 8 lines with high and partial resistance, *Lr10* genes in combination with *Lr39* (*Lr41*) and one sample with a combination of *Lr10* + *Lr16* genes were identified. The obtained result are consistent with the pedigree, according to which donors with these genes were used to create the studied lines. This material is a valuable source for the wheat breeding for resistance to leaf rust, identified genetically.

Key words: winter wheat, introgressive lines. wild relatives, rust resistance, Lr genes.

Introduction. An analysis of the literature revealed that many parent forms of winter wheat introgressive lines are sources of certain leaf rust resistance genes. Based on the introgressive lines pedigree, it was assumed that certain known leaf rust resistance genes may be present in the synthetic breeding lines of KazRIAPG.

At the previous research stages, it was shown that the new lines had a high degree of juvenile resistance, and in the field artificial infection they revealed a high level of resistance.

This work purpose was to analyze the introgressive lines of KazRIAPG breeding winter wheat of molecular markers linked to the Lr9, Lr10, Lr13, Lr16, Lr19, Lr24, Lr34, Lr39 (41), Lr62 genes and select promising lines containing one or more genes resistance to rust.

Materials and methods. The main material was 26 introgressive lines of winter wheat obtained from the wild relatives crossing (*Triticum militinae, T.kiharae, T. timopheevii, T. polonicum, Aegilops cylindrica, Ae.triaristata (Ae. Neglecta Req. Ex Bertol)* varieties susceptible to leaf rust (Bezostaya 1, Zhetysu, Erythrospermum 350, Karlygash, Steklovidnaya 24) Kazakhstan and Russia breeding, as well as 30 lines with varieties. An analysis of the literature revealed that many parental forms of the introgressive winter wheat lines are sources of certain leaf rust resistance genes. Leaf rust resistance genes found in the genealogy of synthetic lines are shown in Table 1. Based on their genealogy, it was suggested that certain known leaf rust resistance genes may be present in the KazRIAPG breeding synthetic lines. In particular, synthetics may contain the *Lr10* + *Lr34* genes from the commercial cvs Bezostaya 1 [1]; *Lr13* - from cvs Zhetysu, cvs Steklovidnaya 24 [2]; *Lr16* - from cvs Karlygash [3]; *Lr 41* - from the species *A.cylindrica*

[4] and *Lr 62* - from the species *Ae.triaristata* (*Ae. Neglecta* Req. Ex Bertol) [5, 6] respectively (table 1). Of the 80 leaf rust resistance genes described in the gene symbol cataloge (DNA markers have been identified for 50%). From literature sources, we selected well-known DNA markers linked to leaf rust resistance genes: *Lr9, Lr10, Lr13, Lr16, Lr19, Lr24, Lr34, Lr39 (41), Lr62.*

Table 1 – Characterization of parental forms of introgressive winter wheat lines by the presence of leaf rust resistance genes

Variety, view	Resistance genes to three types of rust			Literature
	Yr	*Lr*	*Sr*	
Bezostaya 1	*Yr18*	*Lr3a+Lr10+Lr34*	*Sr5*	[1]
Zhetysu		*Lr13*	*Sr8b+Sr5*	[2]
Erythrospermum 350			*Sr8b+Sr5*	[2]
Karlygash		*Lr16*	*Sr11*	[2]
Steklovidnaya 24		*Lr3a+Lr13?*		[2]
T.timopheevii		*Lr18+Lr50+Lr52+LrTt+LrTt2*	*Sr36+Sr37+Sr40*	[3]
Ae.cylindrica		*Lr39 (Lr41)*		[4]
Ae.triaristata (*Ae. neglecta Req. ex Bertol*)	*Yr 42*	*Lr 62*		[5,6]

Primers were selected based on literature data [7-16], their nucleotide sequences are presented in table 2.

Table 2 – Specific DNA marker primers closely linked to wheat leaf rust resistance genes

Gene	Chromo-some	Marker type	Primer Name	Primer sequence (5′–3′)	Amplification Product, p.o.	Source
Lr9	6BL	STS	J13	F: TCCTTTTATTCCGCACGG CGG	1100	[7]
				R: CCACACTACCCCAAAGAGACG		
Lr10	1AS	RFLP/ STS	2245/ Lr10-6/r2	F: GTGTAATGCATGCAGGTTCC	310	[8-9]
				R: AGGTGTGAGTGAGTTATGTT		
Lr13	7BS	SSR	WMC474	F: ATGCTATTAAACTAGCATGTGTCG	120	[10]
				R: GTGCAAACATCATTCCTGGTA		
Lr16	2BS	SSR	Xwmc 764	F: CCTCGAACCTGAAGCTCTGA	180	[11]
				R: TTCGCAAGGACTCCGTAACA		
Lr19	7DL	STS	Gb	F: CATCCTTGGGGACCTC	130	[12]
				R: CCAGCTCGCATACATCCA		
Lr24	3D	SCAR	$SCS1302_{609}$	F: CGCAGGTTCCAAATACTT TTC	607	[13]
				R: CGCAGGTTCTACCTAATGCAA		
				R: TCATCGACGCTAAGGAGGACCC		
Lr34	7DS	STS	csLV34	F: GTTGGTTAAGACTGGTGATGG	150/229	[14]
				R: TGCTTGCTATTGCTGAATCG		
Lr39 (41)	2DS	SSR	GDM35	F: CCT GCT CTG CCC TAG ATA CG	190/280	[15]
				R: ATG TGA ATG TGA TGC ATG CA		
Lr62	6AS		Opw 7.2	F: CAGGAGCATAGTCATACTTGGG	700	[16]
				R: CTGGACGTCAACAATGGC		

Next, primers were synthesized to the loci of the selected DNA markers on the H-16 DNA/RNA/LNA oligonucleotide synthesizer (Germany), according to the instructions attached to the device. This work was carried out on the basis of the Molecular Biology and Genetic Engineering Laboratory NIIPBB. Wheat DNA was isolated from leaves of 4-5 day old seedlings according to the Dellaporta S.L. method [17]. *Lr* genes were identified using the polymerase chain reaction (PCR) method with primers marking the genes *Lr9, Lr10, Lr13, Lr16, Lr19, Lr24, Lr34, Lr39* (41) and *Lr62*. The reaction composition was selected according to the annotation attached to the enzyme and the characteristics of primers. PCR conditions are given in original sources. Almost isogenic lines of the Thatcher cultivar with the indicated leaf rust resistance genes were used as positive controls for determining known genes, and ddH2O as a negative control.

The production of specific DNA regions was carried out in a Termocycler-Pro thermal cycler (Eppendorf, Germany). Identification of the PCR product was carried out using electrophoresis in 1.5% agarose gel (iNtRON, Biotechnology Grade). The amplified fragments were separated in an electrophoretic chamber (Scie-Plas, UK) in TBE buffer with ethidium bromide addition for 1.5 hours at an electric field voltage of 80 V. Analysis of the electrophoresis was carried out using a Mini BIS Pro gel-documenting system, Israel »With software Gel Capture and Gel Quant Express. The amplified fragment lengths were determined in comparison with DNA markers "100 bp DNA Ladder" (Invitrogen Corporation).

Results and discussion. Molecular genetic screening of synthetic winter wheat lines revealed no markers of highly effective leaf rust resistance genes *Lr9, Lr19* and *Lr24*. According to the pedigree, these resistance genes presence is impossible in the studied samples. Since, *Lr9* gene was introgressed into wheat from *Ae.umbellulatum*, and *Lr19* gene was transferred to common wheat from *Agropyron elongatum* and is located on the 7DL chromosome. The stem rust resistance gene *Sr25* is also located in this translocation [18]. The *Lr24* gene is transferred to the common wheat genome from *Ag. elongatum* at least twice: by translocation in the interspecific hybrid [19] and by induction of homeologic mating [20]. These wild relatives (*Ae. Umbellulatum, Agropyron elongatum*) were not involved in the winter wheat synthetics crossing, which allows us to confirm the absence of these genes in studied lines.

It is known that *Lr10* gene is localized on chromosome *6BL* and in studied lines its source is Bezostaya 1 variety of common wheat [1]. In addition, Lr10 gene is one of the most widely represented in Russian varieties [21]. To identify Lr10 gene, we used the marker F1.2245/*Lr10-6*/r2 [8-9]; the molecular size of amplification product with these primers is 310 bp. This marker is the most used for screening wheat in Western European countries. As a result, a marker linked to the *Lr10* gene was identified in 24 of the 56 analyzed samples (figure 1, table 3). The *Lr10* gene currently belongs to the genes group with surpassed efficiency, which is most likely due to its massive use in breeding, both in Russia and Kazakhstan, and abroad. Most of the above lines had a high susceptibility degree in the field and in the seedling phase, which does not deny this gene presence. The *Lr10* gene marker was not detected in individual lines (table 3), although one of their parental forms was the same Bezostaya 1. Despite the fact that the *Lr10* gene is currently ineffective, it has been shown that its combination with other *Lr*-genes can increase the resistance level [23].

Table 3 – Analysis of synthetic winter wheat lines of the KazRIAPG breeding for the presence of leaf rust resistance genes

Name	Pedigree	Presence of (+) resistance gene (s):					
		Lr 10	*Lr13*	*Lr16*	*Lr 34*	*Lr39 (41)*	*Lr 62*
1	2	3	4	5	6	7	8
RFP-1-1	(Bezostaya 1 x *Ae.triaristata*) x Karlygash			+			
RFP-3-1	Bezostaya 1 x *Ae.cylindrica*	+					
RFP-4-1	(Bezostaya 1 x *T.militinae*) x *T.militinae*-6	+					
RFP-5-1	(Bezostaya 1 x *T.militinae*) x *T.militinae*-9	+					
RFP-6-1	(Bezostaya 1 x *T.militinae*) x *T.militinae*-4	+					
RFP-9-1	Zhetysu x *T.timopheevii*		+				
RFP-12-1	Zhetysu x *T.militinae*		+				

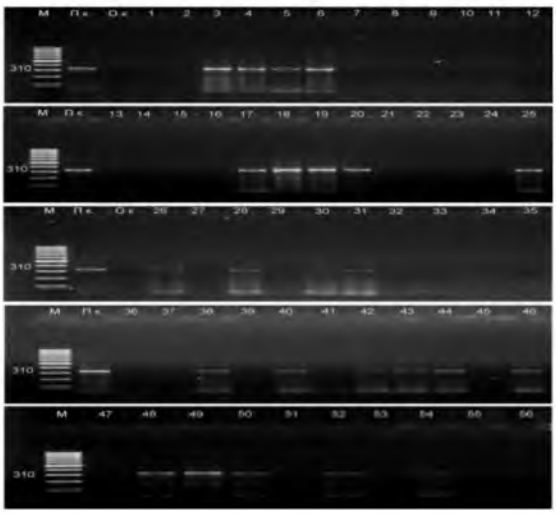
Continuation of table 3

1	2	3	4	5	6	7	8
RFP-14-2	Zhetysu x *T.timopheevii*		+				
RFP-17-2	Erythrospermum 350 x *T.kiharae*	+					
RFP-18-2	Bezostaya 1 x *Ae.cylindrica*	+				+	
RFP-20-2	(Bezostaya 1 x *T.militinae*) x *T.militinae*-9	+					
RFP-21-1	(Bezostaya 1 x *Ae.triaristata*) x Karlygash	+					+
RF-22-2	Zhetysu x *T.militinae*		+				
ЭР350хт2	Erythrospermum 350 x *T.kiharae-2*	+					
1630-2	(Erythrospermum 350x*Ae.cylindrica Host*) x Bezostaya 1	+				+	
1630-5	(Erythrospermum 350 x *Ae.cylindrica H*)x Bezostaya 1	+					
1630-272	(Erythrospermum 350x *Ae.cylindrica Host*) x Bezostaya 1	+					
1634-1	(Bezostaya 1 x *Ae.triaristata Wild*) x Bezostaya 1	+					
1716-42	(Bezostaya 1 x *Ae.cylindrica H.*) x Karlygash	+		+			
1716-45	(Bezostaya 1 x *Ae.cylindrica H.*) x Karlygash			+			
1716-61	(Bezostaya 1 x *Ae.cylindrica H.*) x Karlygash	+					
1717-210	(Bezostaya 1 x *Ae.cylindrica H.*)x Steklovidnaya 24					+	
1717-450	(Bezostaya 1 x *Ae.cylindrica H.*)x Steklovidnaya 24	+				+	
1718-55	(Bezostaya 1 x *Ae.cylindrica H.*) x Erythrospermum 350	+				+	
1718-58	(Bezostaya 1 x *Ae.cylindrica H.*) x Erythrospermum 350	+					
1718-60	(Bezostaya 1 x*Ae.cylindrica Host*) x Erythrospermum 350					+	
1718-62	(Bezostaya 1 x*Ae.cylindrica Host*) x Erythrospermum 350	+				+	
1719-5	(Bezostaya 1 x *Ae.triaristata W.*) x Karlygash	+					
1719-9	(Bezostaya 1 x *Ae.triaristata W.*) x Karlygash	+					
1719-10	(Bezostaya 1 x *Ae.triaristata W.*) x Karlygash	+					
1720-3	(Bezostaya 1 x *Ae.triaristata W.*) x Erythrospermum 350	+					
2040-1	(Bezostaya 1 x*Ae.cylindrica Host*) x Erythrospermum 350	+				+	
2044-3	(*T.polonicum* x Zhetysu) x Zhetysu		+				

The *Lr13* gene is one of the most widely represented in wheat varieties worldwide and is closely linked to the Ne2m hybrid necrosis gene. Until recently, this symptom was used as a morphological marker. McIntosh et al. [23] revealed a close linkage of the *Lr13* gene with *Lr23* in a number of Australian and Indian wheat varieties. Currently, several microsatellite markers are used to identify the *Lr13* gene (Xgwm630, WMC474, Xksm58, Xstm773b). There is conflicting information in the literature about the individual markers effectiveness. More often they are characterized as uninformative. We used the WMC47 microsatellite marker for screening winter wheat introgressive lines. This marker was selected in hybrid combination analysis Leichardt x WAWHT2071 and is used in breeding programs in Australia [10]. As a result of PCR, the Lr13 gene presence was established in Zhetysu x *T.timopheevii* lines; Zhetysu x *T.militinae*; Zhetysu x *T.kiharae*; Zhetysu x *T.militinae*-2 (table 3, figure 2), created on Zhetysu variety basis, which, according to published data, is the carrier of the *Lr13* and Sr8b + Sr5 genes [2]. Earlier, on the phytopathological test basis, it was suggested that the cvs Steklovidnaya 24 may be the carrier of the *Lr13* gene [2]. But, during molecular screening, a specific fragment of amplification was not observed either in cvs Steklovidnaya 24 or in other lines created with the participation of this variety.

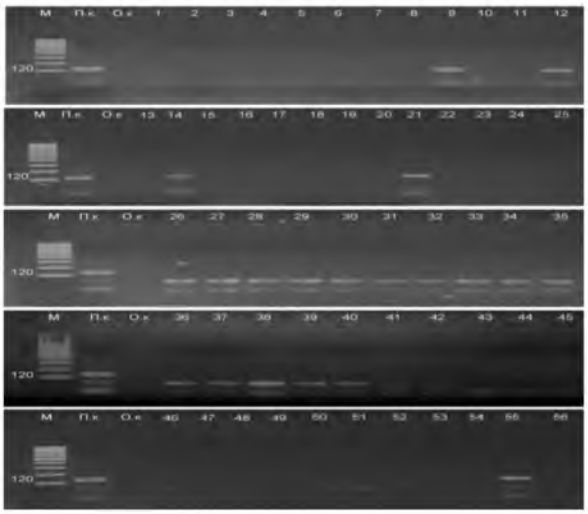
The next *Lr16* gene is located on the 2BS chromosome, the source of the gene is Selkirk (Canada), testing line of the Thatcher variety is RL6005. To identify the *Lr16* gene carriers, we selected the Xwmc764 marker, developed on the SSR marker basis. This marker is localized on the long arm of chromosome 2B and flanks the locus of the Lr16 gene at a 1.9 cm distance [11]. It is found in samples with the *Lr16* gene as an amplification product with a molecular weight of 180 bp. K.Nazari et al. [2] previously identified this gene in cvs Karlygash winter wheat variety by postulating resistance genes. In

our experiments, 10 synthetic lines were created with the cvs Karlygash variety participation. Among them, the *Lr16* gene was identified only in lines 231-1 (Bezostaya 1 x *Ae.triaristata*) x Karlygash,



M - marker (100 bp DNA Ladder), P.C. - positive control (Thatcher line with Lr10), N.c. - negative control (H2O), 1-56 - synthetic lines of winter wheat (samples name and origin are shown in table 3)

Figure 1 – Amplification products using a pair of primers 2245/ *Lr10-6* /r2 to a diagnostic marker linked to the sheet rust resistance gene *Lr10*



M - marker (100 bp DNA Ladder), P.K. - positive control (Thatcher line with Lr13), N.c. - negative control (H2O), 1-56 - synthetic lines of winter wheat (samples name and origin are shown in table 3)

Figure 2 – Amplification products using a pair of WMC474 primers for a diagnostic marker linked to the sheet rust resistance gene *Lr13*

1716-42 and 1716-45 (table 3), representing selections from one combination. For most of the lines, the presence of this gene was not detected.

The *Lr39* (Lr41) gene was transferred to common wheat from five samples *Ae. tauschi* of various geographical origin, as well as from *Ae.cylindrica*. The *Lr39* gene is predominantly found in North American varieties, and the Thunderbolt variety was the first to introduce this gene. In our experiments, many synthetic winter wheat lines (20 lines) were obtained with the participation of *Ae.cylindrica*, in connection with which we suggested that these lines can carry the *Lr39* gene (*Lr41*). Currently, the identification of this gene is possible based on various molecular markers analysis. According to published data, the SSR marker to the GDM35 locus is the most diagnostic compared to other markers [16].

In this regard, in this experiment, we used a pair of primers for the SSR locus of GDM35 marker. When using GDM35 primers in lines with a functional allele of *Lr39* gene (*Lr41*), an amplification product with a molecular weight of 190 bp is detected, with a non-functional allele from 214 to 280 bp. (depending on the genotype), heterozygous - both of these products. The distance between the marker and the gene is estimated at 1.9 cM [15]. As a result, PCR in 8 lines (1718-2, 1630-2, 1717-210, 1717-450, 1718-55, 1718-60, 1718-62, 2040-1) out of 56 detected a diagnostic marker linked to the *Lr39* gene (*Lr41*). At the same time, at the previous research stages (table 4), it was shown that the marked lines had a high degree of juvenile resistance, and in the field, against the background of artificial infection, they revealed a high age resistance level.

Table 4 – Field resistance of promising lines of winter wheat from 2 nurseries to fungal diseases

Sample Name	Winter hardiness / regrowth, score	Spikeling date	Type and degree of damage, score /%					The development degree of leaf spot,%	
			yellow rust		leaf rust				
			1	2	1	2	3	1	2
1	2	3	4	5	6	7	8	9	10
1630-2	5/4	19.05.16	1/5	2/5	1/10	2/10	3/30	5	10
1630-4	5/5	17.05.16	0	0	2/10	3/20	3/40	10	10
1630-5	5/5	17.05.16	0	0	3/20	4/30	4/60	0	0

Continuation of table 4

1	2	3	4	5	6	7	8	9	10
1630-10	5/5	15.05.16	0	0	2/10	3/10	3/20	5	10
1630-272	5/5	13.05.16	2/5	2/10	1/5	2/5	4/30	5	10
1634-1	5/5	15.05.16	2/20	2/20	1/5	2/5	2/10	5	10
1675-72	4/5	18.05.16	2/10	2/10	2/10	3/10	3/10	10	20
1675-72	4/5	18.05.16	2/5	4/10	2/5	2/5	3/10	10	10
1680-4	4/5	20.05.16	0	2/5	1/5	1/5	2/10	10	10
1680-9	4/5	16.05.16	2/5	2/5	1/5	1/5	1/5	5	10
1712-8	5/5	16.05.16	0	0	2/5	2/5	2/10	5	10
1712-36	5/5	17.05.16	2/10	2/10	2/20	4/30	4/40	20	20
1716-42	5/5	18.05.16	0	0	2/30	3/50	4/60	10	10
1716-45	5/5	16.05.16	2/20	3/20	2/20	3/20	4/30	20	20
1716-61	5/5	16.05.16	0	1/5	1/5	1/5	3/30	40	40
1717-210	5/4	17.05.16	0	0	0	0	2/10	20	30
1717-450	4/5	17.05.16	0	0	0	0	0	40	20
1718-55	4/5	19.05.16	0	0	0	0	0	0	5
1718-58	4/5	19.05.16	0	0	2/20	3/40	4/60	20	30
1718-60	4/5	16.05.16	0	0	0	0	0	0	0
1718-62	5/5	17.05.16	0	0	0	0	0	20	20
1719-3	5/5	16.05.16	0	0	0	0	0	10	10
1719-5	4/5	17.05.16	0	0	2/10	3/10	4/30	20	30
1719-9	4/5	15.05.16	0	0	1/5	3/10	4/20	10	10
1719-10	5/5	15.05.16	0	0	2/20	3/20	4/50	10	10
1719-215	5/5	14.05.16	0	0	2/10	4/30	4/60	30	40
1720-3	5/5	14.05.16	1/5	1/5	2/20	2/40	3/60	20	20
1721-69	5/5	15.05.16	2/20	4/30	1/5	1/5	1/5	10	20
2040-1	5/5	15.05.16	3/10	3/10	3/10	3/10	3/10	20	20
2044-3	5/5	17.05.16	0	1/5	0	0	0	20	20
Steklov. 24	5/5	05.05.16	3/30	4/40	3/20	4/30	4/60	40	40

Thus, the results of molecular screening are consistent with phytopathological data. In addition, fragments with a non-functional allele (from 214 to 280 bp) were amplified in 32 lines, and null alleles of the *Lr39* gene (*Lr41*) were detected in 16 lines.

The *Lr34* gene belongs to the group of genes that provide partial (partial) resistance, which is characterized by horizontal stability indicators: an increase in the latent period, a decrease in the number of pustules and their size. This gene was first described by Dyck in 1977 [22], and later the same author established its localization in chromosome 7D [24]. Further studies showed that the *Lr34* gene is located on the short arm of chromosome 7D [25]. In addition, it was found that it is genetically inseparable from the APR gene *Yr18*, associated with moderate resistance to yellow rust [26, 27]. The cosegregation of this gene with the powdery mildew resistance gene Pm38 was revealed [28]. The locus is also associated with resistance to the yellow dwarf virus of barley *Bdv1* [29]. The main morphological manifestation of the *Lr34* gene is leaf tips necrosis [30]. In the world, a lot of work has been done on monitoring wheat collections for the presence and elucidation of the allelic state of the *Lr34* gene using molecular genetic markers. In Russian varieties, the *Lr34* gene is widely used from Bezostaya 1, which is widely used in wheat hybridization. Despite the fact that *Lr34* gene efficiency in Russia has been lost, it has been shown that its combination with other race-specific genes, for example *Lr13*, significantly increases the level of field resistance [22].

The codominant STS marker csLV34 was derived from the RFLP marker and is closely linked to the Lr34 locus (0.4 cM) [11]. This marker is most commonly used for screening wheat around the world. When using csLV34 primers, an amplification fragment with a molecular weight of 150 bp indicates the presence of a functional gene allele, 229 bp - to a non-functional allele, and of both fragments to a heterozygous state. We used this marker to identify the *Lr34* gene in the studied winter wheat lines. As a result, a specific amplification product with a molecular weight of 150 bp found only in the control line with the *Lr34* gene. The absence of a functional allele of the *Lr34* gene in the studied lines is probably due to the use of various genetic material and the characteristics of the selection process. To more accurately answer this question, it is necessary to conduct additional studies using other markers. Although, an amplification product of 229 bp in size, indicating the presence of a non-functional allele of the gene, was observed in many synthetic lines (25 lines) obtained with the participation of Bezostaya 1 (table 3).

Translocation with the *Lr62* gene is transmitted to common wheat from *Ae. neglecta* Req. ex Bertol (*Ae.triaristata*) and can be localized on chromosome 6AS. The yellow rust resistance gene *Yr42* is also located in this translocation. The *Lr62* gene is effective against a wide range of *Puccinia triticina* pathotypes in southern Africa and western Canada [5, 6]. To identify the *Lr62* gene, the only molecular marker Opw 7.2 was proposed [16]; however, this marker is still validated for use in MAS schemes. However, in our experiments, we used the Opw 7.2 marker for molecular screening of 9 synthetic lines obtained from the crossbreeding of common wheat varieties with *Ae.triaristata* (*Ae. Neglecta* Req. Ex Bertol). It should be noted that we do not have a line — the positive control of *Lr62* — and its presence was judged by the presence of a diagnostic fragment (700 bp) during PCR analysis. As a result, fragments of different sizes and not only those declared as diagnostic are amplified. A 700 bp amplicon, described as a diagnostic fragment by A. Eksteen [16], was observed at line 231-2 (Bezostaya 1 x *Ae.triaristata*) x Karlygash (table 3). However, the visualization in the agarose gel was not clear enough, which does not allow us to conclude that this gene is present in this line, and the results should be regarded as preliminary. Moreover, this work requires continuation, in particular, conducting a phytopathological test and PCR analysis using an effective molecular marker, as well as a cytogenetic study of the transfer of genetic material from *Ae. neglecta* Req. ex Bertol (*Ae.triaristata*) into created synthetic lines.

Of particular importance are these research results due to the fact that high elements content found highly resistant genotypes among previously identified sources.

Conclusions. The *Lr10* gene was identified for 24 (out of 56) lines, in the genealogy of which there is a Bezostaya 1 variety (also a carrier of this gene) and was not found for 9 lines. For the *Lr13* gene, its presence was noted in 4 lines created with the Zhetysu cvs participation. The *Lr16* gene was detected for Karlygash cvs and in 3 out of 10 lines that have it in pedigree, these are numbers 231-1; 1716-42 and 1716-45. For 8 out of 20 lines with the presence of *Ae.cylindrica* in the origin, a marker linked to the *Lr39* gene (*Lr41*) was detected, and in combination with cvs Erythrospermum 350 and Steklovidnaya 24.

It is known that a combination of several leaf rust resistance genes in one genotype can provide more reliable and long-lasting protection due to the genetic resistance basis expansion. In 8 lines with high and partial resistance (1718-2, 1630-2, 1717-210, 1717-450, 1718-55, 1718-60, 1718-62, 2040-1), *Lr10* genes in combination with *Lr39* (*Lr41*), (1716-42) was one sample –with a combination of *Lr10* + *Lr16* genes was revealed. Thus, the results of molecular screening are consistent with phytopathological data [32].

Thus, using molecular approaches, the genetic basis of winter wheat synthetic lines for leaf rust resistance was characterized. This material is a valuable source for the wheat breeding on leaf rust resistance.

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ГЕНІ ТАТҚА ТӨЗІМДІ ЖАБАЙЫ ТҰҚЫМДАСТАРДЫҢ ҚАТЫСУЫ АРҚЫЛЫ КҮЗДІК БИДАЙДЫҢ ИНТРОГРЕССИВТІК СЫЗЫҚТАРЫН ИДЕНТИФИКАЦИЯЛАУ

Аннотация. Қазақстан және Ресей селекциясыныц жабайы тұқымдастармен будандастыру арқылы алган күздік бидайдың 26 интрогрессивті сызыгымен (*Triticum militinae, T.kiharae, T.timopheevii, T.dicoccoides, Aegilops cylindrica, Ae.triaristata* (*Ae. neglecta Req.ex Bertol*) жапырақты татқа тұрақты және сезімтал сұрыптар («Безостая 1», «Жетісу», «Эритроспермум 350», «Қарлыгаш», «Стекловидная 24»), сонымен қатар табиги және жасанды фон негізінде аталық және аналық түрлеріне қатысты дифференциалданган 30 сызық. Lr гендерін жүйелеу праймермерлермен, Lr9, Lr10, Lr13, Lr16, Lr19, Lr24, Lr34, Lr39(41) және Lr62 генін маркерлейтін полимеразалы тізбек (ПТР) әдісін қолдану арқылы жүргізілді.

24 сызыққа қатысты Lr10 гені жүйеленді, олардың ішінде аталық және аналық түрлерінде «Безостая 1» (сонымен қатар ген тасымалдаушысы) кездесті және 9 сызыққа қатысты анықталмады. Жетісу сұрыбының қатысуы арқылы алынган сызықта Lr13 гені анықталды. «Қарлығаш» сұрыбына арналган Lr16 гені анықталды және 10 сызықтың 3-де дәлелденді, педигриде 231-1 (Безостая 1 х *Ae.triaristata*) х Қарлыгаш); 1716-42 (Безостая 1 х *Ae.cylindrica H.*) х Қарлыгаш)) және 1716-45 (Безостая 1 х *Ae.cylindrica H.*) х Қарлыгаш)). Ae.cylindrica қатысуы арқылы алынган 20 сызықтың 8-інде Lr39 генімен (Lr41) байланысқан маркер анықталды және «Эритроспермум 350» және «Стекловидная 24» комбинациясында қанықкан.

Бір генотиптегі бірнеше жапырақты татқа төзімді гендердің комбинациясы төзімділіктің генетикалық негізінің кеңеюіне байланысты негұрлым сенімді және ұзақ мерзімді қоргауды қамтамасыз ететіні белгілі. Жогары және ішінара тұрақтылыққа ие сегіз сызықта (1718-2, 1630-2, 1717-210, 1717-450, 1718-55, 1718-60, 1718-62, 2040-1) бастапқы көзі Ae.cylindrica болып саналатын Lr39 (Lr41) үйлесімінде Lr10 гені анықталды. Ae.cylindrica тән болып келетін Lr10+Lr16 ген комбинациясы бір үлгіде (1716-42) анықталды. Молекулалық скрининг Lr10 тұрақтылық гені 56 сызықтың 24-нде («Безостая 1» сортында және ондағы будандарда), 4 үлгіде Lr13 («Жетісу» сорты және оның қатысуы арқылы алынган сызықтар), 3 үлгіде Lr16 (Қарлыгаш сорты және оның қатысуы негізінде алынган сызықтар), 8 үлгідегі Lr39 (Lr41) комбинациясында Ae.cylindrica арқылы «Эритроспермум 350» және «Стекловидная 24» сорттарымен қанықкан, сәйкесінше жогары және ішінара тұрақтылыгы бар Lr10 гені негізінде Lr39 (Lr41) және бір үлгідегі Lr10 + Lr16 генінің жиынтыгы бар үлгі (Безостая 1 х *Ae.triaristata*) х Қарлығаш-2 Lr10 Lr62.

Сары тотқа төзімді генотиптер арасында жапырақты тотқа тұрақты сызықтар белгілі болды: 1719-3, 1719-5, 1719-9, 1719-10, 1719-215, Lr10 комбинациясында асылтұқымды Ae.triaristata (Yr 42) бар.

Осылайша, молекулалық тәсілдерді қолдана отырып, күздік бидайдың синтетикалық жолдарының генетикалық негізі олардың жапырақты татқа төзімділігі негізінде сипатталды. Бұл материал табиги және жасанды иммунологиялық талдаулар нәтижесінде алынган және бидай-эгилопс KZ231 негізінде бидай сұрыбын құру арқылы дәлелденген бидай сұрыпталуының құнды көзі болып саналады (10.23.2019 ж. №199 / 025.4 патенттік өтінім).

Түйін сөздер: күздік бидай, интрогресивті үлгілер, жабайы тұқымдас, тат ауруына тұрақтылық, Lr-гендері.

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ИДЕНТИФИКАЦИЯ ИНТРОГРЕССИВНЫХ ЛИНИЙ ОЗИМОЙ ПШЕНИЦЫ С УЧАСТИЕМ ДИКИХ СОРОДИЧЕЙ ПО ГЕНАМ УСТОЙЧИВОСТИ К РЖАВЧИНЕ

Аннотация. 26 интрогрессивных линий озимой пшеницы, полученные от скрещивания дикорастущих сородичей (*Triticum militinae, T.kiharae, T.timopheevii, T.dicoccoides, Aegilops cylindrica, Ae.triaristata* (*Ae. neglecta Req.ex Bertol*), с умеренно устойчивыми и восприимчивыми к листовой ржавчине сортами (Безостая 1, Жетысу, Эритроспермум 350, Карлыгаш, Стекловидная 24) селекции Казахстана и России, а также 30 линий с насыщением сортов дифференцированы ранее на естественном и искусственном фоне относительно родительских форм. Идентификацию генов Lr осуществляли с использованием метода полимеразной цепной реакции (ПЦР) с праймерами, маркирующими гены Lr9, Lr10, Lr13, Lr16, Lr19, Lr24, Lr34, Lr39(41) и Lr62.

Ген Lr10 идентифицирован для 24 линий, в родословной которых присутствует сорт Безостая 1 (также носитель этого гена) и не обнаружен для 9 линий. Для гена Lr13 отмечено его присутствие в линиях, созданных с участием сорта Жетысу. Ген Lr16 выявлен для сорта Карлыгаш и у 3-ех из 10 линий, имеющих его в педигри, это номера 231-1 (Безостая 1 х *Ae.triaristata*) х Карлыгаш); 1716-42 (Безостая 1 х *Ae.cylindrica H.*) х Карлыгаш) и 1716-45 (Безостая 1 х *Ae.cylindrica H.*) х Калыгаш)). Для 8 из 20 линий с присутствием в происхождении *Ae.cylindrica* детектирован маркер, сцепленный с геном Lr39 (Lr41), причем в комбинациях с насыщением Эритроспермум 350 и Стекловидная 24.

Известно, что сочетание нескольких генов устойчивости к листовой ржавчине в одном генотипе может обеспечивать более надежную и продолжительную защиту вследствие расширения генетической основы устойчивости. В 8 линиях с высокой и частичной устойчивостью (1718-2, 1630-2, 1717-210, 1717-450, 1718-55, 1718-60, 1718-62, 2040-1) выявлены гены Lr10 в сочетании с Lr39 (Lr41), источником которого является Ae.cylindrica. Выявлен один образец (1716-42) с комбинацией генов Lr10+Lr16, для всех характерно присутствие Ae.cylindrica. Молекулярный скрининг показал наличие гена устойчивости Lr10 у 24 из 56 линий (как у сорта Безостая 1 и гибридов с ним), у 4 образцов – Lr13 (сорт Жетысу и линии с его участием), у 3 образцов - Lr16 (сорт Карлыгаш и линии с его участием), у 8 образцов – Lr39 (Lr41) с присутствием в происхождении *Ae.cylindrica* в комбинациях с насыщением сортов Эритроспермум 350 и Стекловидная 24, соответственно с высокой и частичной устойчивостью выявлены гены Lr10 в сочетании с Lr39 (Lr41) и один образец с комбинацией генов Lr10+Lr16 и образец (Безостая 1 х *Ae.triaristata*) х Карлыгаш-2 Lr10 Lr62.

Среди генотипов, устойчивых к желтой ржавчине, дополнительно устойчивости к листовой ржавчине отмечены формы: 1719-3, 1719-5, 1719-9, 1719-10, 1719-215, имеющие в своей родословной *Ae.triaristata* (Yr 42) в комбинации с Lr10.

Таким образом, с использованием молекулярных подходов была охарактеризована генетическая основа синтетических линий озимой пшеницы по устойчивости к листовой ржавчине. Данный материал является ценным источником для селекции пшеницы на устойчивость к листовой ржавчине, как было показано в результате иммунологического анализа на естественном и искусственном фоне и подтверждено созданием нового сорта на пшенично-эгилопсной основе KZ231 (Заявка на патент №2019/025.4 от 23.10.2019 г.).

Ключевые слова: озимая пшеница, интрогрессивные линии, дикие сородичи, устойчивость к ржавчине, Lr-гены.

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