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RESEARCH ARTICLE

Screening of the Bread Wheat Varieties for the Leaf Rust Resistance Gene *Lr34/Yr18/Sr57/Pm38/Bdv1*

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Abstract:

Background:

The allelic composition of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1,* which is associated with resistance to leaf rust in varieties of bread wheat (*Triticum aestivum* L.), has been investigated.

Methods:

Three DNA markers were used to determine the allelic state of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1*: the co-dominant molecular genetic markers *cssfr5* and *csLV34* and the microsatellite marker *Xgwm295*.

Results:

Among 32 cultivars evaluated for resistance to leaf rust, 4 were highly resistant, 26 were resistant and 2 were moderately susceptible. Using the co-dominant marker *cssfr5* based on the detection of the polymorphic state of one of the exons of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1*, the *Lr34(+)* allele, which confers resistance to leaf rust, was found in 25% of the studied varieties. The coincidence between the results obtained with the markers *cssfr5* and *csLV34* was 84.5%.

Conclusion:

The data of the conducted molecular genetic analysis were supplemented by observations of the resistance of the studied varieties to leaf rust in the field. The obtained data can be used in breeding programs to develop new varieties and breeding lines with leaf rust resistance.

Keywords: Bread wheat, leaf rust, Marker-assisted selection, Polymerase chain reaction, Resistance, Polymorphic state.

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1. INTRODUCTION

Leaf rust caused by the pathogenic fungus *Puccinia triticina* Erikss. is one of the most widespread wheat diseases in Ukraine, as well as globally [1, 2]. According to FAO, during rust epiphytotic, wheat crop losses can amount up to 40%. The most effective method of plant protection is to develop resistant varieties. At present, more than 80 genes for leaf rust resistance have been identified in the genome of wheat, and its relatives [3], and molecular markers are commonly used to identify them [4]. Leaf rust resistance genes were designated as *Lr* in the catalogue of gene symbols [3].

Wheat breeding for resistance to fungal diseases is based on a combination of genes of race-specific (vertical) and race-nonspecific (horizontal) resistance, which makes it possible to ensure a high level of resistance over a long period of time [5]. Most genes that control race-specific resistance to rusts remain effective only for several years [6]. Race-nonspecific rust resistance is commonly moderate, but this type of resistance, which is not based on specific recognition between the host and the pathogen, proves to be durable. Horizontal resistance to

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rust manifests at the adult plant stage as the adult plant resistance (APR) [7]. Unlike most *Lr* genes, which provide resistance for only a few years, the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene retains its effectiveness for many seasons [8]. The *Lr34/Yr18/Sr57/Pm38/Bdv1* gene is race-nonspecific and provides moderate resistance to leaf rust in adult plants [1, 9]. This gene was first discovered in 1977 and mapped to chromosome 7D [10]. The *Lr34* gene also provides moderate resistance to other biotrophic pathogens: yellow rust caused by *P. striiformis* Westend. f. sp. *tritici* (in this case, it is referred to as the *Yr18* gene) [11], stem rust caused by *Puccinia graminis* Pers. (as *Sr57*) and powdery mildew caused by *Blumeria graminis* (DC.) (DC.) Speer f. sp. *tritici* (referred to as *Pm38*) [12]. The *Lr34/Yr18/Sr57/Pm38* pleiotropic set possessed by Bezostaya 1 currently represents an important target for wheat breeding because it is now amenable to molecular selection [2]. It is also associated with resistance to yellow dwarf barley mosaic virus (the *Bdv1* gene) [13]. Besides, the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene is also closely linked to the *Ltn1* gene for leaf tip necrosis [14]. The phenotypic expression of the *Ltn1* gene was used by Singh *et al*. [15] to detect the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene, but the multigenic effect on the overall manifestation of flag leaf tip necrosis and unstable *Ltn1* expression under different conditions may lead to ambiguous results [16].

There is considerable interest in developing effective methods for detecting wheat disease resistance genes. Microsatellite DNA markers *Xgwm295* and *Xgwm122*0 were among the first markers used to identify the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene [17]. However, these markers have not been widely used because of their low efficiency. In addition, two other markers, *Xswm10* and *csLVMS1* [18], were used to detect the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene. However, practical application of these markers was restrained by a difficulty in routine differentiation of alleles due to the small difference between the amplicons of 206 bp and 208 bp for the marker *Xswm10* [12], and similarly, between the amplicons of 224 bp and 226 bp for the marker *csLVMS1* [19]. The most widely used molecular marker for *Lr34/Yr18/Sr57/Pm38/Bdv1* gene is *csLV34* [11]. This co-dominant marker was actively used to detect alleles of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene in bread wheat samples from Europe, Australia, Canada, and the USA [20, 21].

The nucleotide sequence of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene is 11805 bp, contains 24 exons and encodes a protein with a length of 1401 amino acid residues [22]. The alleles at this locus differ by a single nucleotide substitution in exon 4, a trinucleotide deletion in exon 11, and a single nucleotide substitution in exon 12 [8, 23]. Based on the locus structure, a co-dominant molecular genetic marker *cssfr5* was developed and proved to be the most effective [8]. However, according to the literature, there are some varieties carrying a characteristic resistance allele of the marker *Lr34(+)* in exon 11, but they do not possess the leaf rust resistance [7].

The aim of our study was to compare the efficiency and reliability of widely used indirect molecular markers *csLV34* and *Xgwm295* to the locus-specific marker *cssfr5* of the Lr*34/Yr18/Sr57/Pm38/Bdv1* gene in a sample of modern wheat varieties originating from different climatic zones and to evaluate the possible impact of the gene on leaf rust resistance in the field.

2. MATERIALS AND METHODS

To assess the resistance of wheat samples and identify carriers of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene, Ukrainian varieties developed at the Institute of Plant Physiology and Genetics at the National Academy of Sciences of Ukraine (IPPG NASU) under the supervision of Prof. V.V. Morgun, varieties of the Myronivka Institute of Wheat named after V.M. Remeslo at the National Academy of Agrarian Sciences of Ukraine (NAASU) (hereinafter MIW), the Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation (PBGI), the NAASU National Science Centre "Institute of Agriculture" (IA), the company "Roden 10", as well as some varieties from other countries have been used.

Isolation of total DNA from plant material. Wheat grains were used as plant material for DNA isolation. The isolation of total DNA was performed by the CTAB method [24].

Polymerase chain reaction (PCR). The reaction mixture consisted of the following components: specific primers (Table **1**) with the concentration of 0.5 μm, 2 μl of 10 × Reaction buffer B, 2.0 mM $MgCl_2$, 0.2 mM each deoxyribonucleotide-3-phosphate (Thermo Fisher Scientific), 0.5 units of DreamTaq polymerase ™ DNA Polymerase (Thermo Fisher Scientific), 50-100 ng of total DNA, deionized water Milli-Q (Merck Millipore) adjusted to a final volume of 20 μl. Amplification reactions were performed in a Mastercycler gradient (Eppendorf) thermal cycler.

Table 1. Characteristics of DNA markers associated with the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene.

S.No	Marker	Primers and Their Sequence	Amplicon Size, bp
1.	*Xgwm295*	F: GTGAAGCAGACCCACAACAC R:GACGGCTGCGACGTAGAG	256 [25] 254 250
2.	*csLV34*	csLV34F: GTTGGTTAAGACTGGTGATGG csLV34R: TGCTTGCTATTGCTGAATAGT	229 [21] 150 [20]
3.	*cssfr5*	L34DINT9F: 5'TTGATGAAACCAGTTTTTTTTCTA3' L34MINUSR:5'TATGCCATTTAACATAATCATGAA3' L34SPF:5'GGGAGCATTATTTTTTTCCATCATG3' L34DINT13R2:5'ACTTTCCTGAAAATAATACAAGCA3'	751 [8] 523

Leaf rust resistance of the wheat varieties was assessed at the Experimental Agricultural Production fields of IPPG NAS of Ukraine. Evaluation of the extent of the leaf rust development was performed using the integrated scale of resistance of cereals against *P. triticina* (Table **2**).

The amplification programs used were the following (according to [8, 20, 21, 25] with modifications). For the marker *cssfr5*: initial denaturation at 94°C for 3 min, 8 cycles: denaturation at 94°C, annealing at 68 (-1) °C for 30 s, elongation at 72 °C for 50 s, then 26 cycles: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 50 s, final elongation at 72°C for 5 min. For the marker *csLV34*: initial denaturation for 3 min at 94°C, 38 cycles: denaturation at 94°C for 45 s, annealing for 30 s at 55°C,

elongation at 72°C for 1 min, final elongation at 72°C for 5 min. For the marker *Xgwm295*: initial denaturation at 94°C for 3 min, 35 cycles: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 1 min, final elongation at 72°C for 5 min.

DNA electrophoresis. PCR products were separated in an agarose gel in SB buffer (5 mM $Na_2B_4O_7$, pH 8.5) and LB buffer (10 mM $Li_2B_4O_7$, pH 8.5). GeneRulerTM DNA Ladder Mix (Thermo Fisher Scientific), Quick-Load® Purple 50 bp DNA Ladder (BioLabs) and pUC19 / MspI (Thermo Fisher Scientific) molecular weight markers were used to determine the product size. Amplicons were visualized in ultraviolet light (LKB Transilluminator Macrovue 2011), registered with the Canon EOS 600D photosystem. The images were processed with a GIMP editor and GelAnalyzer. RunSAFE, a non-mutagenic fluorescent reagent that provides instant visualization of DNA bands under UV illumination of agarose gels, was used for electrophoresis of amplicons from PCR with the *csLV34* marker.

To analyze the amplification patterns for the marker *Xgwm295*, we used an Agilent 2100 bioanalyzer, based on a lab-on-a-chip technology and is designed for the analysis of proteins and nucleic acids. With this bioanalyzer, the analysis time was reduced to 40 minutes. The bioanalyzer allows to separate DNA fragments with an accuracy of up to 3 base pairs.

The leaf rust resistance of bread wheat plants was assessed in the dynamics (throughout the course of the disease development). The main field assessment was carried out at the period of maximum disease development – at the milk stage [26].

The severity of the disease was determined by the area of the affected surface of leaves covered with spots or intensity of other symptoms by the formula [26]:

$$R = \frac{100 \times \sum ab}{N},$$

where R is the disease severity, %;

a–number of plants with disease symptoms;

b–intensity of infection, %;

N–total number of plants.

The resulting data were analyzed by one-way analysis of variance.

3. RESULTS

Initially, to identify the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene, we used the *Xgwm295* microsatellite marker, located on chromosome 7D. Among the cultivars analyzed using the microsatellite marker *Xgwm295,* the following alleles were detected: 250 bp, 254 bp, 256 bp. The cultivars 'Bogdana', 'Zolotokolosa', 'Smuglyanka' carried the 250-bp allele. The 254-bp allele was detected in the varieties 'Podolyanka' and 'Natalka'. For the rest of the varieties, the 256-bp allele was identified (Fig. **1**). The previous research reported that the presence of the 254-bp allele of the microsatellite marker *Xgwm295* indicates the presence of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1* [12].

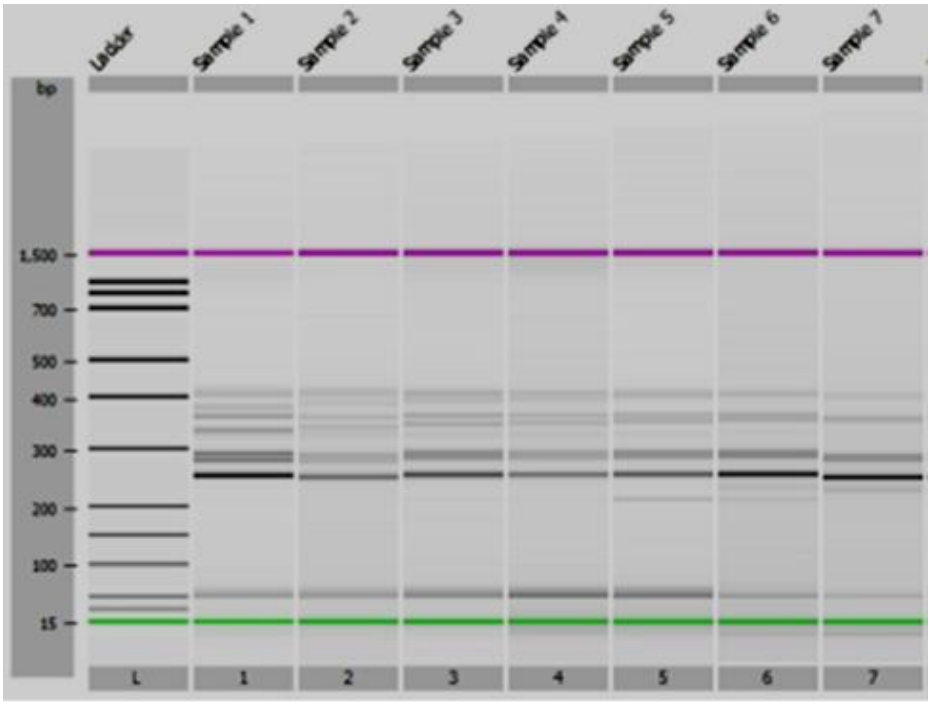

Fig. (1). Electrophoregram of DNA amplification products on the microsatellite marker *Xgwm295* obtained on an Agilent 2100 bioanalyzer for wheat varieties: lane 1 – 'Natalka', 2 – 'Zolotokolosa', 3–6 – 'Podolyanka', 7 – 'Smuglyanka', L – 100 bp ladder.

In addition, the co-dominant marker *csLV34* was used. A sufficiently high degree of association of this marker with the resistance gene *Lr34/Yr18/Sr57/Pm38/Bdv1* has been established [22] and verified [27, 28]. This marker detects two alleles: *csLV34a* with amplified fragments of 229 bp, indicating an absence of wheat resistance to leaf rust, and *csLV34b* – the 150-bp fragments, indicating the presence of such resistance [29]. These alleles have a deletion size of 79 bp. Only one of the two alleles of the *csLV34* marker was detected in all studied wheat samples (Fig. **2**). The cultivars polymorphic for this trait were not detected in the samples under study. The resistance-associated *csLV34b* allele (150 bp) was detected in 5 wheat varieties: 'Zolotokolosa', 'Mironovskaya-30', 'Panna', 'Glenlea', and 'Nedra', which accounted for 15.6% of the samples studied [30]. The *csLV34a* allele was present in 27 samples or 84.4% of the total number of 32 varieties tested. The full list of the cultivars analyzed is presented in Table **2**.

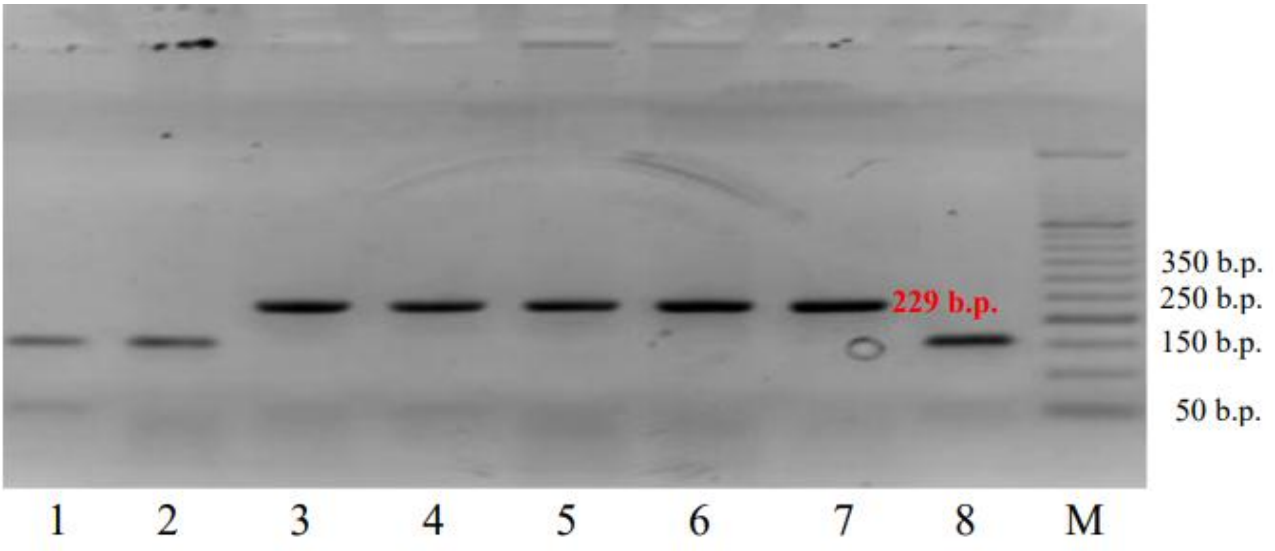

Fig. (2). Electrophoregram of DNA amplification products with use of the marker *csLV34* for wheat varieties: 1, 2 – 'Zolotokolosa', 4 – 'Natalka', 5, 6, 7 – 'Pivna', 8 – 'Mironovskaya-30', M - Quick-Load® Purple 50 bp DNA Ladder.

Table 2. The integrated scale of leaf rust resistance of cereals [26].

Score	Disease Symptoms	Degree of Resistance
9	No visible symptoms	Immune
8	Rare chlorotic and necrotic spots with very small uredinia are observed. The infection intensity 1-5%	Highly resistant
7-6	Small and moderate pustules in chlorotic and necrotic spots, the infection intensity 6-10% and 11-15%	Resistant
5	Uredinia with the infection intensity 16-25%, Light chlorosis and/or necrosis can be observed	Moderately susceptible
4-3	Moderate to large uredinia with the infection intensity from 26-40% to 41-65%, light chlorosis is possible	Susceptible
2	Large uredinia, infection intensity 66-90%	Highly susceptible
1	Large merged uredinia, infection intensity 91-100%	Extremely susceptible

Table 3. Comparison of the allelic composition of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1* in common wheat varieties according to the three DNA markers used

S.No	Cultivar	Size of the Amplified Fragments for *Xgwm295* (bp)	Allelic State of the *Lr34* Gene	Size of the Amplified Fragments for *csLV34* (bp)	Allelic State of the *Lr34* Gene	Size of the Amplified Fragments for *cssfr5* (bp)	Allelic State of the *Lr34* Gene
Ukrainian cultivars							
Cultivars from the Institute of Plant Physiology and Genetics of the NAS of Ukraine							
1	'Bogdana'	250	-	229	-	523	-
2	'Vesnyanka'	*	-	229	-	523	-
3	'Volodarka'	*		229	-	751	+
4	'Zimoyarka'	256	-	229	-	523	-

(Table 3) contd.....

S.No	Cultivar	Size of the Amplified Fragments for *Xgwm295* (bp)	Allelic State of the *Lr34* Gene	Size of the Amplified Fragments for *csLV34* (bp)	Allelic State of the *Lr34* Gene	Size of the Amplified Fragments for *cssfr5* (bp)	Allelic State of the *Lr34* Gene
5	'Zolotokolosa'	250	-	150	+	523	-
6	'Kievskaya-Ostistaya'	256	-	229	-	523	-
7	'Lasunya'	256	-	229	-	523	-
8	'Natalka'	254	+	229	-	523	-
9	'Niva-Kievshciny'	256	-	229	-	523	-
10	'Novokiivska'	256	-	229	-	523	-
11	'Pereyaslavka'	256	-	229	-	523	-
12	'Pivna'	256	-	229	-	523	-
13	'Podolyanka'	254	+	229	-	751	+
14	'Smuglyanka'	250	-	229	-	523	-
15	'Sonechko'	256	-	229	-	523	-
16	'Favoritka'	256	-	229	-	523	-
17	'Khutoryanka'	256	-	229	-	523	-
18	'Yatran-60'	256	-	229	-	751	+
Cultivars from Remeslo Institute of Wheat of the NAAS of Ukraine							
19	'Krizhinka'	*		229	-	751	+
20	'Mironovskaya-808'	256	-	229	-	523	-
21	'Mironovskaya-30'	256	-	150	+	751	+
Cultivars of Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation - of the NAAS of Ukraine							
22	'Bilyava'	256	-	229	-	523	-
23	'Oksana'	256	-	229	-	523	-
24	'Panna'	256	-	150	+	751	+
Cultivars of the National Scientific Centre "Institute of Agriculture of the NAAS of Ukraine"							
25	'Nedra'	256	-	150	+	751	+
Cultivar of the "Roden10" farm							
26	'Torchynska'	256	-	229	-	523	-
Foreign cultivars							
27	'Aranka'	256	-	229	-	523	-
28	'Glenlea'	256	-	150	+	751	+
29	'Granny'	256	-	229	-	523	-
30	'Triso'	256	-	229	-	523	-
31	'Tybalt'	256	-	229	-	523	-
32	'Federer'	256	-	229	-	523	-

"*" In the samples did not detect the presence of the fragment of amplification using these primers.

Subsequently, another study was performed to identify the allelic state of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene using the molecular genetic marker *cssfr5*. Fig. (**3**) shows an electrophoregram with the amplified DNA fragments for the wheat varieties analyzed with the marker *cssfr5*.

Out of the whole set of 32 varieties under study, only 8, 'Podolyanka', 'Krizhinka', 'Yatran-60', 'Volodarka', 'Mironovskaya-30', 'Panna', 'Nedra' and 'Glenlea', contained the *Lr34(+)* allele, which constituted 25% of the total number of the varieties tested (Table **2** and Fig. **3**). 26 of them were selected in Ukraine (Table **3**).

The allele *Lr34(+)* of the marker *cssfr5* was identified only in 7 Ukrainian varieties, which is 27%, and in one out of 6 foreign varieties ('Glenlea'). The results obtained *cssfr5* were not completely identical to those with the marker *csLV34* in 27 varieties, and the degree of coincidence proved to be 84.5%. Apparently, there are differences in the use of these two markers to identify the gene *Lr34/Yr18/Sr57/Pm38/Bdv1*.

Our data showed that in the studied wheat cultivars, resistance to leaf rust was between 5 and 8 points. The cultivars 'Natalka', 'Smuglyanka', 'Zolotokolosa' and 'Favoritka' had a resistance score of 8 and were highly resistant to leaf rust. The plants had almost no signs of the disease and the intensity of the lesion was as low as up to 5%. The other 26 varieties were less resistant, with the intensity of the lesion ranging from 5.6% to 14.8%. The most susceptible to the disease were varieties 'Khutoryanka' and 'Mironovskaya-808'. The total intensity of lesion per plant for the variety 'Khutoryanka' was 16.7%, and for the variety 'Mironovskaya-808' – 24.2% (Table **4**). Our data indicate (Table 4) that out of 32 studied cultivars, 4 cultivars were highly resistant, 26 – resistant and 2 – moderately susceptible.

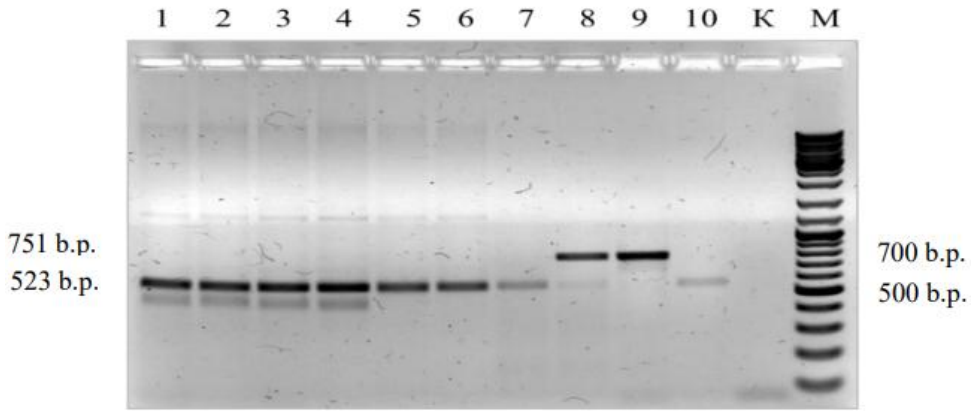

Fig. (3). Electrophoregram of PCR results using the marker *cssfr5*: 1, 2 – 'Natalka', 3, 4 – 'Zolotokolosa', 5, 6 – 'Pivna', 7 – 'Kievskaya-Ostistaya', 10 – 'Lasunya'. Lanes 1-7, 10 - samples of wheat type *Lr34(-*); 8, 9 – 'Volodarka', 8-9 - wheat variety *Lr34(+);* K - negative control (without DNA); M - GeneRuler ™ DNA Ladder Mix.

Table 4. Resistance of wheat varieties to leaf rust.

S.No	Cultivar	Leaf Rust		
		Index of Resistance	The Intensity of Lesion, %	Level of esistance or Susceptibility
Ukrainian cultivars				
Cultivars from the Institute of Plant Physiology and Genetics of NAS of Ukraine				
1	'Bogdana'	7-6	6.7	Resistant
2	'Vesnyanka'	7-6	14.8	Resistant
3	'Volodarka'	7-6	12.6	Resistant
4	'Zimoyarka'	7-6	9.4	Resistant
5	'Zolotokolosa'	8	4.7	Highly Resistant
6	'Kievskaya-Ostistaya'	7-6	8.4	Resistant
7	'Lasunya'	7-6	7.3	Resistant
8	'Natalka'	8	5.0	Highly Resistant
9	'Niva-Kievshchiny'	7-6	11.2	Resistant
10	'Novokiivska'	7-6	9.4	Resistant
11	'Pereyaslavka'	7-6	8.2	Resistant
12	'Pivna'	7-6	12.6	Resistant
13	'Podolyanka'	7-6	8.5	Resistant
14	'Smuglyanka'	8	3.4	Highly Resistant
15	'Sonechko'	7-6	10.0	Resistant
16	'Favoritka'	8	4.3	highly Resistant
17	'Khutoryanka'	5	16.7	Moderately susceptible
18	'Yatran 60'	7-6	10.5	Resistant
Cultivars from Remeslo Institute of Wheat of NAAS of Ukraine				
19	'Krizhinka'	7-6	9.6	Resistant
20	'Mironovskaya-808'	7-6	12.2	Resistant
21	'Mironovskaya-30'	5	24.2	Moderately susceptible
Cultivars of Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation- of NAAS of Ukraine				
22	'Bilyava'	7-6	7.2	Resistant
23	'Oksana'	7-6	6.3	Resistant
24	'Panna'	7-6	7.8	Resistant
Cultivar of National Scientific Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine"				
25	'Nedra'	7-6	9.0	Resistant
Cultivars from the "Roden10" farm				
26	'Torchynska'	7-6	7.4	Resistant
Foreign cultivars				

(Table 4) contd.....

S.No	Cultivar	Leaf Rust		
		Index of Resistance	The Intensity of Lesion, %	Level of esistance or Susceptibility
27	'Aranka'	7-6	5.6	Resistant
28	'Glenlea'	7-6	4.3	Resistant
29	'Granny'	7-6	5.8	Resistant
30	'Triso'	7-6	6.9	Resistant
31	'Tybalt'	7-6	6.2	Resistant
32	'Federer'	7-6	6.1	Resistant

The study of wheat cultivars using molecular markers revealed that several varieties contained the gene *Lr34/Yr18/Sr57/Pm38/Bdv1*, which confers resistance to leaf rust. It was important to evaluate the effect of this gene on the intensity of infestation of wheat samples by leaf rust in field trials. Using the marker *cssfr5*, the varieties under examination were divided into two groups: those containing the resistance-associated allele *Lr34(+),* and those containing susceptibility-associated allele *Lr34(-).* Eight of the test varieties had the *Lr34(+)* allele, while the *Lr34(-)* allele was detected in most varieties.

According to the statistical analysis conducted, the correlation between resistance/lack of resistance of varieties to leaf rust in the field and the allelic composition of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene was insignificant. The level of significance of differences p> 0,05; F_{fact} = 1.519903 <F_{teor} = 3.369016; p = 0.237539. The average values of lesion intensity were: 8.65 ± 4.62 in varieties which did not have the *Lr34(+)* allele and 9.31 ± 2.64 in varieties that contained the *Lr34(+)* allele. Thus, no significant effect of the presence of the *Lr34(+)* allele on the manifestation of resistance to leaf rust in the varieties studied has been established.

4. DISCUSSION

The difference in the results for the microsatellite marker *Xgwm295*, co-dominant molecular genetic markers *csLV34* and *cssfr5* can be explained by the fact that the locus *Xgwm295* and the locus *csLV34* are not part of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene, therefore markers *Xgwm295* and *csLV34* are less accurate than *cssfr5*.

The comparative analysis has also been conducted to analyze the obtained data on the allelic state of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1* in Ukrainian varieties with the outcomes of previous studies. Detection of the allelic composition of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene in 181 cultivars of Ukrainian breeding was performed by Kozub *et al*. [31]. The authors showed that the resistance associated allele *Lr34* + was identified in 42.8% of the varieties analyzed, and in varieties of PBGI breeding its frequency was 57.8%.

Previous research shows that the gene *Lr34/Yr18/Sr57/Pm38/Bdv1* originated from an old Italian variety 'Rieti' [29]. Italian breeder Strampelli created the 'Mentana', 'Ardito', 'Ballila', and 'Villa Glori' varieties using the breeding line selected from 'Rieti', a high-yielding Dutch variety 'Wilhelmina' and a precocious, resistant to lodging Japanese variety 'Akagomughi', which had been used for crossbreeding. Thus, the 'Frontana' variety was obtained by using the variety 'Mentana', the carrier of the *Lr34* + allele [29, 32]. From the variety 'Frontana', the gene was translocated into wheat varieties in the United States, Canada, and other countries. The donor of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene in European wheat varieties, including 'Bezostaya 1', is almost certainly the 'Ardito' variety [32, 33]. Our research results confirm the hypothesis that the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene was introduced into Ukrainian wheat varieties from 'Bezostaya 1'. The cultivars 'Panna', 'Mironovskaya-30', 'Krizhinka', which contain the allele *Lr34 (+),* originate from 'Bezostaya 1' (Fig. **4**) [32].

The high resistance of varieties with the *Lr34(-)* allele to the leaf rust pathogen can be explained by the presence of other resistance genes, which were beyond the scope of this work. Although no significant effect of the *Lr34(+)* allele on leaf rust resistance has been established, it might be appropriate to conduct a further study to elaborate a possible role of the gene on yellow rust and powdery mildew as well as spot blotch resistance (the latter one has been reported in the literature [34]) under the field conditions in Ukraine.

The results of our research are in good agreement with the previously published studies of the allelic state of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene [1, 7, 25]. However, for some varieties, the results obtained in this research were different, for instance, the varieties 'Nedra' and 'Podolyanka' were previously reported to carry the susceptible allele of the gene [5, 31] while, according to our results, they carry the allele of resistance (Table **3**). As for the 'Pivna' variety, it was previously found that the variety was polymorphic [7, 31] while in this study, only the sensitivity allele was detected (Table **3**). Such differences may be due to the heterogeneity of the seed material of wheat varieties of Ukrainian breeding used for different studies. Some varieties of Ukrainian and foreign breeding were analyzed using molecular markers of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene for the first time in this study. The results of our field research confirm the findings previously obtained: the allelic state of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene did not correlate with the field resistance of wheat varieties of Ukrainian origin as it has already has been shown by the independent trials under different conditions [34]. It can be summarized that although resistance-associated allele of the gene is widespread among wheat cultivars of Ukrainian origin its potential has not been yet fully exploited due to lack of appropriate tools (that is, molecular markers). Therefore the development of the breeding lines with a combination of the resistance-associated allele of *Lr34/Yr18/Sr57/Pm38/Bdv1* and other leaf rust resistance genes may be recommended as a follow-up to this study.

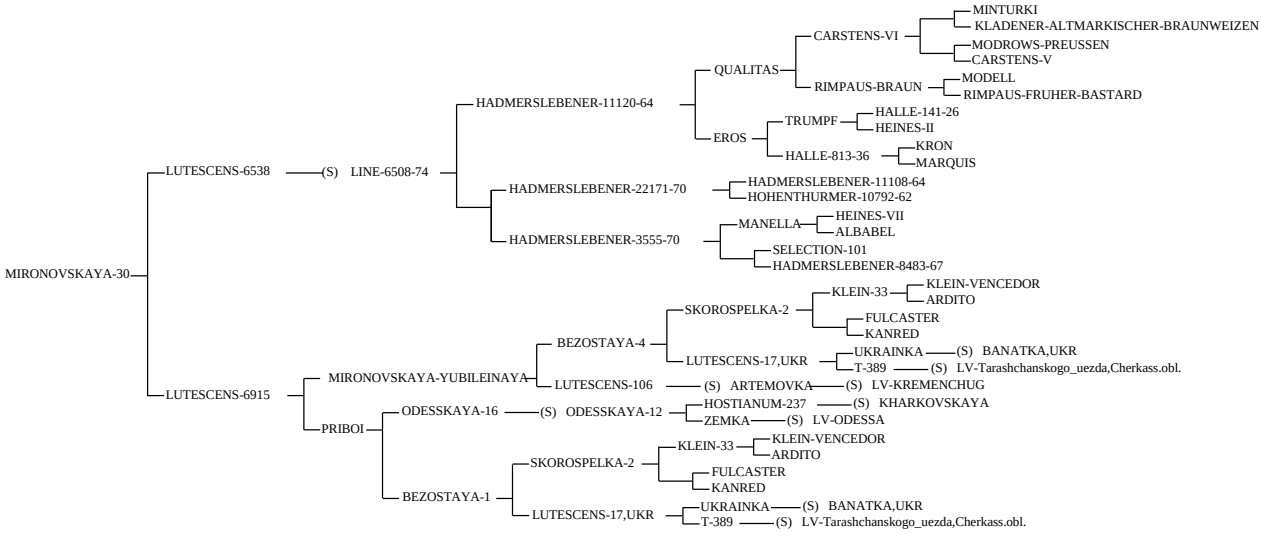

Fig. (4). Pedigree for 'Mironovskaya-30' [32].

CONCLUSION

DNA markers *csLV34*, *Xgwm295* and *cssfr5* were used to evaluate alleles of the *Lr34/Yr18/Sr57/Pm38/Bdv1* gene in Ukrainian and foreign bread wheat cultivars. In the sample, two alleles of the *csLV34* marker have been detected: *csLV34a* and *csLV34b*; three alleles for the microsatellite marker *Xgwm295*: 250 bp, 254 bp and 256 bp and finally, two alleles, *Lr34(+)* and *Lr34(-),* of the gene-localized marker *cssfr5*. The coincidence between the results obtained with the markers *cssfr5* and *csLV34* was 84.5%. According to our research, eight wheat samples contained the *Lr34(+)* allele associated with leaf rust resistance which is 25% of the total number of wheat varieties studied. Our results have also confirmed the hypothesis that the source of the gene *Lr34/Yr18/Sr57/Pm38/Bdv1* in the varieties of Ukrainian breeding is 'Bezostaya 1'.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data are represented in (Figs. **1**-**4**) Tables **1** – **4**.

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None.

CONFLICT OF INTEREST

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