the permanent habitats, the number dynamics, as well as the fauna of the intermediate hosts themselves.

So, due to the deforestation and the expansion of the planting-soil fields, rodents have also moved to different directions by changing their permanent locations. In the places they go, they infect other animals by spreading the helminth eggs and they themselves are infected with other species of helminths which are inherent in new environment. Other animals feeding with these infected rodents are infected with new species of helminths (6).

Since such cases last a long time, the helminth fauna with a new species composition of domestic carnivores has formed and has acquired its modern status.

Thus, the analysis of the results obtained from the carried out studies has found that the anthropogenic factors are primarily affect the formation of helminth fauna, the formation of parasitic focuses, the increasing number of pathogen helminthes assuming epizootic and epidemiological significance which include into composition of the helminth fauna and, ultimately, infection of humans and domestic animals with parasitic diseases. Therefore, the anthropogenic factors that adversely affect the formation of the helminth fauna of animals should be more clearly defined and should always be the focus of attention.

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Resistance of winter wheat cultivars to leaf rust in conditions of the Central Caucasus

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Abstract

The main problem of wheat immunity to leaf rust is the loss of efficiency of most *Lr*-genes. The decrease in efficiency is associated with microevolutionary processes within the population and the emergence of new virulent phytopathogen races that can overcome previously efficient resistance genes. The article presents the results of the phytopathological test and marker analysis of the selected material of winter wheat for resistance to the leaf rust pathogen (*Puccinia recondita* Rob.ex Desm.f. *sp. tritici*.). The object of the research was 20 cultivar samples of various ecological and geographical origins. DNA was isolated from the leaves of 10-day-old wheat germs. Molecular markers were used for the following genes: *Lr9* (SCS5), *Lr10* (Fi.2245/Lr10-6/r2), *Lr19/Sr25* (SCS265), *Lr20/Sr15* (STS638), *Lr24/Sr24* (Sr24#12), *Lr34/Sr57* (csLV34), *Lr37/Sr38/Yr17/Pch2/Cre5* (Ventriup/LN2), *Lr41* (GDM35), *Lr47* (PS10). Using molecular markers, the studied wheat varieties did not reveal the highly

and partially effective genes *Lr9*, *Lr19/Sr25*, *Lr24/Sr24*, *Lr41*, and *Lr47* in Russia, and the ineffective gene *Lr20/Sr1*.

As a result of molecular screening, it was found that the List 25 variety had *Lr37* genes; the Mif variety had *Lr10* genes; the Eltan variety had *Lr10* genes; the Markola variety had *Lr34* genes; the Malvina variety had *Lr26* genes; the Tvorets variety had *Lr10* genes; the DB 1/05 variety had *Lr10* genes; the Evklid variety had *Lr10* genes; the Sumai aut variety had *Lr34* genes; the Lebidka odes'ka variety had *Lr34* genes; the Solara variety – *Lr34*; the Zhiva variety – *Lr10*, *Lr34*. When comparing the results of marker analysis with field resistance to leaf rust, the resistant type of reaction to infection (R) was shown by the cultivars: Battum, Eltan, Evklid, Areal, and Solara; the susceptible type of reaction (S) was noted in the cultivars Markola and Mallyska; the medium susceptible type of reaction (MS) – in the cultivars Lebidka odes'ka and Tvorets.

Introduction

Wheat leaf rust (*Puccinia recondita* Rob.ex Desm.f. *sp. tritici*.) refers to one of the harmful diseases, with a large distribution area in all grain-producing regions of Russia. The most effective and environmentally-friendly method of combating this disease is breeding for disease resistance.

In this regard, the creation of genetically diverse source material of winter wheat, carrying effective resistance genes to leaf rust, is one of the main tasks of breeding. To increase the effectiveness of breeding programs on the resistance of varieties to diseases, various methods, including molecular and genetic, are successfully used.

The use of molecular genetic markers makes it possible to identify effective resistance genes in varieties and hybrids, accelerate the selection of target genotypes, and increase the efficiency of the breeding process. The work objective of this paper is to identify resistance genes (*Lr*-genes) to leaf rust in the breeding material of winter wheat.

Materials and methods

The material for our investigation was 20 varieties of winter soft wheat of various ecological and geographical origins (Russia, Ukraine, Slovakia, USA). A phytopathological test and molecular markers for *Lr* resistance genes were used.

In the germinal phase, the juvenile resistance of soft wheat samples to leaf rust was evaluated. For infection, the Krasnodar population of *P. tritici* was used, which was represented by a mixture of pathogen isolates isolated from different wheat varieties cultivated in the Krasnodar Agricultural Research Institute in 2020. Reproduction of the fungus culture was performed following the method of L.A. Mikhailova [5].

The resistance assessment was carried out using wheat germs (plumule sheath phase). The studied samples were sprayed with an aqueous suspension of the studied pathogens. Leaf rust resistance was evaluated 8-10 days after inoculation using a scale: where 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 – pustules of different types on the same leaf, chloroses and necrosis are observed [4]. Plants with reaction type 0, 1, 2 were classified as resistant, 3, 4, X – as susceptible. The plant reaction type to the pathogen introduction was determined on days 8-10 after infection following the international scale. The following genes were identified using molecular markers: *Lr9* (SCS5), *Lr10* (Fi.2245/Lr10-6/r2), *Lr19/Sr25* (SCS265), *Lr20/Sr15* (STS638), *Lr24/Sr24* (Sr24#12), *Lr34/Sr57* (csLV34), *Lr37/Sr38/Yr17/Pch2/Cre5* (Ventriup/LN2), *Lr41* (GDM35), *Lr47* (PS10)) [1, 6]. DNA was isolated from the leaves of 10-day-old wheat germs following the Dorokhov and Kloke method [2]. DNA amplification was carried out in a reaction mixture according to the protocols proposed in the literature. The resulting amplified fragments were separated by electrophoresis in horizontal agarose gels in a 1×TBE buffer.

Results

Disease resistance is one of the main factors affecting plant productivity. It is determined by the presence or absence of the corresponding genes and their state. The use of molecular markers in practical selection is denoted by the term MAS (marker-assisted selection) [1, 2]. The basic principle of the MAS is to identify the close linkage between a marker and a gene that controls a trait, and use the marker-trait association for practical purposes, for example, to create new varieties and breeding lines [4, 5]. Once the marker-trait association is established, the creation of new genotypes can proceed with the involvement of already traditional breeding methods. Markers of economically valuable genes allow for more reliable selection not only by phenotype but also by genotype [6, 7].

To date, the wheat gene catalog contains information on 69 *Lr*-genes responsible for resistance to leaf rust [4, 8]. However, one of the main problems of wheat immunity to the disease is the short-lived effectiveness of most *Lr*-genes. The decrease in the efficiency of genes is associated with microevolutionary processes within the population and the emergence of new virulent phytopathogen races that can overcome the resistance of the variety. As a result, many of the known *Lr*-genes become ineffective [3].

The objects of the study were 20 varieties of winter wheat. Table 1 shows the degree of virulence of cultivars to leaf rust in natural field conditions and one of the main indicators of productivity – the weight of grain per spike (Table 1).

Table 1

Resistance of wheat samples to the causative agent of leaf rust

No.	Name	Natural background	Weight of grain per spike, g	Origin
1	List 25	MR (medium resistant)	1.0	Ukraine
2	Battum	R (resistant)	1.8	USA
3	Mif	M (mediate)	0.9	Russia
4	Eltan	R	2.0	USA
5	Timiryazevskaya 150	MR	0.9	Russia
6	Markola	S (susceptible)	0.8	Slovakia
7	Verita	MR	2.0	Slovakia
8	Mallyska	S	0.8	Slovakia
9	Malvina	MR	2.0	Slovakia
10	Tvoretz	MS (medium susceptible)	1.2	Russia
11	DB 1/05	MR	1.5	Russia
12	Evklid	R	2.0	Ukraine
13	Adele	MR	0.9	Russia
14	Sumai out	MR	1.2	China
15	Areal	R	1.5	Ukraine
16	Lebidka odes'ka	MS	1.4	Ukraine
17	Praskoviya	MR	1.1	Russia
18	Solara	R	2.1	Slovakia
19	Zhiva	MR	0.6	Russia
20	Etyud	MR	0.8	Russia

Using molecular markers, the studied wheat varieties did not reveal the highly and partially effective genes *Lr9*, *Lr19/Sr25*, *Lr24/Sr24*, *Lr41*, and *Lr47* in Russia, and the ineffective gene *Lr20/Sr15* (Fig. 1). The *Lr9* gene is localized in the 6B chromosome, virulence to the gene is rare, the gene is highly effective. The *Lr10* gene is isolated from hexaploid wheat and located on the 1AS chromosome. After expression in transgenic wheat plants, *Lr10* provided increased resistance to leaf rust [9].

Fig. 1 PCR electrophoregrams with gene markers *Lr9* (a), *Lr19/Sr25* (b), *Lr24/Sr24* (c), *Lr41* (d), *Lr47* (e), and *Lr20/Sr15* (e).

Discussion

The source of the *Lr19*, *Lr24*, and *Lr29* genes is the wheat-grass *Agropyron elongatum*. Very rarely appearing virulent pathotypes do not yet have aggressiveness and do not pose a threat to carriers of this gene. Using the Fi.2245/Lr10-6/r2 marker, the *Lr10* gene was identified in 40% of the cultivars: List 25, Mif, Eltan, Tvorets, DV 1105, Evklid, Zhiva, and Etyud. Using the csLV34 marker, the age resistance gene *Lr34* was detected in 25% of the varieties: Markova, Sumai aut, Lebidushka odes'ka, Solara, and Zhiva.

The *Lr34* gene is located in the short arm (p) of the 7D chromosome and closely linked to the powdery mildew resistance (*Pm38*) and yellow rust resistance (*Yr18*) genes, as well as to the leaf tip necrosis gene (*Ltn1*). *Lr34* belongs to a group of genes that provide resistance to both qualitative and quantitative manifestations (i. e., partial resistance or, in other words, resistance to the type of slow development – slow rusting). This type of resistance is characterized by a longer latent period, a decrease in the number of pustules per leaf surface unit, their size, and the number of spores in the pustules. Using the Ventriup/LN2 marker, the age resistance gene *Lr37* was detected in the List 25 variety. Translocation with the *Lr37* gene is given to soft wheat from *Triticum ventricosum* Ces. (= *Aegilops ventricosa* Tausch.) and localized in the short arm of the 2A chromosome. This translocation also contains the resistance genes to stem (*Sr38*) and yellow (*Yr17*) rust which is effective in Russia. The summary PCR results are presented in Table 2.

As a result of molecular screening, it was found that the List 25 variety had *Lr37* genes; the Mif variety had *Lr10* genes; the Eltan variety had *Lr10* genes; the Markola variety had *Lr34* genes; the Malvina variety had *Lr26* genes; the Tvorets variety had *Lr10* genes; the DB 1/05 variety had *Lr10* genes; the Evklid variety had *Lr10* genes; the Sumai aut variety had *Lr34* genes; the Lebidka odes'ka variety had *Lr34* genes; the Solara variety – *Lr34*; the Zhiva variety – *Lr10*, *Lr34*.

Table 2

Results of the study of wheat varieties for the presence of Lr genes using molecular markers

Name	<i>Lr9</i>	<i>Lr10</i>	<i>Lr19</i>	<i>Lr20</i>	<i>Lr24</i>	<i>Lr34</i>	<i>Lr37</i>	<i>Lr41</i>	<i>Lr47</i>
List 25	–	+	–	–	–	–	+	–	–
Battum	–	–	–	–	–	–	–	–	–
Mif	–	+	–	–	–	–	–	–	–
Eltan	–	+	–	–	–	–	–	–	–
Timiryazevskaya 150	–	–	–	–	–	–	–	–	–
Markola	–	–	–	–	–	+	–	–	–
Verita	–	–	–	–	–	–	–	–	–
Mallyska	–	–	–	–	–	–	–	–	–
Malvina	–	–	–	–	–	–	–	–	–
Tvorets	–	+	–	–	–	–	–	–	–
DB 1/05	–	+	–	–	–	–	–	–	–
Evklid	–	+	–	–	–	–	–	–	–
Adele	–	–	–	–	–	–	–	–	–
Sumai aut	–	–	–	–	–	+	–	–	–
Areal	–	–	–	–	–	–	–	–	–
Lebidka odes'ka	–	–	–	–	–	+	–	–	–
Praskoviya	–	–	–	–	–	–	–	–	–
Solara	–	–	–	–	–	+	–	–	–
Zhiva	–	+	–	–	–	+	–	–	–
Etyud	–	+	–	–	–	–	–	–	–

When comparing the results of marker analysis with field resistance to leaf rust, the resistant type of reaction to infection (R) was shown by the cultivars: Battum, Eltan, Evklid, Areal, and Solara; the susceptible type of reaction (S) was noted in the cultivars Markola and Mallyska; the medium susceptible type of reaction (MS) – in the cultivars Lebidka odes'ka and Tvorets.

The obtained results will be used in breeding programs to create varieties resistant to leaf rust for the conditions of the Central Caucasus.

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