Breeding of winter wheat for resistance to leaf rust in the foothill zone of the Central Caucasus

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Abstract. The decrease in the efficiency of most Lr-genes is associated with microevolutionary processes within the population and the emergence of new virulent races of the phytopathogen, which are capable of overcoming previously effective resistance genes. The article presents the results of a phytopathological test and marker analysis of the selection material of winter wheat for resistance to the causative agent of brown rust (Puccinia recondita Rob.ex Desm f. sp. tritici). The object of research was 20 varieties of different ecological and geographical origin. DNA was isolated from the leaves of 10-day-old wheat seedlings. 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). As a result of molecular screening, it was established that the Lr37 genes were identified in the List 25 cultivar; for the Myth variety - Lr10; for the Eltan variety - Lr10; Markola variety - Lr34; for the Malvina variety - Lr26; the variety Creator - Lr10; for variety DB 1/05 - Lr10; the Evklid cultivar has the Lr10 gene; the variety Sumai aut - Lr34; in the Lebidka odes'ka variety - Lr34; Solara variety - Lr34; the variety Zhiva - Lr10, Lr34. When comparing the results of marker analysis with field resistance to leaf rust, a resistant type of reaction to infection (R) reaction (S) was observed in the Markola and Mallyska cultivars; medium susceptible type of reaction (MS) - in cultivars Lebidka odes'ka and Tvorets.

1. Introduction

Wheat brown rust (Puccinia recondita Rob.ex Desm f. sp. tritici.) Belongs to one of the harmful diseases, with a large distribution area in all grain-growing regions of Russia. The most effective and environmentally friendly method of combating this disease is selection for resistance. In this regard, the creation of a genetically diverse starting material of winter wheat, carrying effective genes for resistance to leaf rust, is one of the main tasks of breeding. In order to increase the effectiveness of breeding programs for the resistance of varieties to diseases, various methods, including molecular genetic ones, are successfully applied. 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 aim of this work is to identify genes of resistance (Lr-genes) to leaf rust in breeding material of winter wheat.

2. Materials and methods
The material for our research was 20 varieties of soft winter wheat of various ecological and geographical origin (Russia, Ukraine, Slovakia, USA). Used a phytopathological test and molecular markers for resistance Lr-genes.

In the seedling phase, the juvenile resistance of soft wheat samples to leaf rust was assessed. For infection, the Krasnodar population of P. triticina was used, represented by a mixture of pathogen isolates isolated from different varieties of wheat cultivated in KNIISH in 2020. The culture of the fungus was propagated according to the method of L.A. Mikhailova [1].

Resistance was assessed using wheat seedlings (first leaf phase). The studied samples were sprayed with an aqueous suspension of the studied pathogens. Assessment of resistance to leaf rust was carried out 8–10 days after inoculation using a scale: where 0 - no symptoms; 0 - necrosis without pustules; 1 - very small pustules surrounded by necrosis; 2 - pustules of medium size, surrounded by necrosis or chlorosis; 3 - pustules of medium size without necrosis, 4 - large pustules without necrosis, X - pustules on the same leaf of different types, chlorosis and necrosis are present [4]. Plants with reaction types 0, 1, 2 were referred to as resistant, 3, 4, X - to susceptible. The type of plant response to the introduction of a pathogen was determined 8–10 days after infection according to an international scale. The following genes were identified using molecular markers:

\[ Lr9 \ (SCS5), \ Lr10 \ (Fi.2245/Lr10-6/i2), \ Lr19/Sr25 \ (SCS265), \ Lr20/Sr15 \ (STS638), \ Lr24/Sr24 \ (Sr24#12), \ Lr34/Sr57 \ (csLV34), \ Lr37/Sr38/Yr17/Pch2/Cre5 \ (Ventriup/LN2), \ Lr41 \ (GDM35), \ Lr47 \ (PS10) \] [1,6]. DNA was isolated from the leaves of 10-day-old wheat seedlings by the method of Dorokhov and Kloke [2]. DNA amplification was carried out in the reaction mixture according to the protocols proposed in the literature. The resulting amplified fragments were separated by electrophoresis in horizontal agarose gels in 1 × TBE buffer.

3. Results and discussion
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 MAS is to identify a close linkage between a marker and a gene that controls a trait and to use the marker-trait association for practical purposes, for example, to create new varieties and breeding lines [3, 4]. After the marker-trait association has been established, the creation of new genotypes can proceed with the involvement of already traditional methods of selection. Markers of economically valuable genes make it possible to more reliably select not only by phenotype, but also by genotype.

To date, the catalog of wheat genes contains information on 69 Lr-genes responsible for resistance to leaf rust [5, 6]. However, one of the main problems of wheat immunity to disease is the short-term effectiveness of most Lr-genes. The decrease in gene efficiency is associated with microevolutionary processes within the population and the emergence of new virulent races of the phytopathogen, which are able to overcome the resistance of the variety. As a result, many of the known Lr-genes become ineffective [7].

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 is the weight of grain per ear (table 1).

| Variety | Natural background | Grain weight per ear, g | Origin |
|---------|--------------------|------------------------|--------|
| List 25 | MR (moderately stable) | 1,0                    | Ukraine |

Table 1. Resistance of wheat samples to leaf rust pathogen
Using molecular markers, the studied wheat varieties did not reveal highly and partially effective genes \( Lr9, Lr19 / Sr25, Lr24 / Sr24, Lr41 \) and \( Lr47 \) in Russia and the ineffective gene \( Lr20 / Sr15 \) (figure 1). The \( Lr9 \) gene is localized on chromosome 6B, virulence to the gene is rare, and the gene is highly efficient. The \( Lr10 \) gene is isolated from hexaploid wheat and is located on chromosome 1AS. When expressed in transgenic wheat plants, \( Lr10 \) provided increased resistance to leaf rust [8, 9].
4. Conclusion

The source of genes Lr19, Lr24, Lr29 is wheatgrass *Agropyron elongatum*. Very rarely appearing virulent pathotypes do not yet possess aggressiveness and do not pose a threat to carriers of this gene. Using the F1.2245 / Lr10-6 / r2 marker, the Lr10 gene was identified in 40% of cultivars: List 25, Myth, Eltan, Creator, DV 1105, Evklid, Zhiva and Etude. Using the csLV34 marker, the age resistance gene Lr34 was identified in 25% of cultivars: Markova, Sumai aut, Lebidushka odes'ka, Solara, and Zhiva. The Lr34 gene is localized in the short arm of chromosome 7D and is closely linked to the genes for resistance to powdery mildew (*Pm38*) and yellow rust (*Yr18*), *as well as the gene for leaf tip necrosis (Ltn1)*. Lr34 belongs to a group of genes that ensure the stability of both qualitative and quantitative manifestation. This type of resistance is characterized by a longer latency period, a decrease in the number of pustules per unit of leaf surface, their size and the number of spores in pustules. Using the Ventriup / LN2 marker, the age resistance gene Lr37 was identified in the List 25 variety. The translocation with the Lr37 gene was transferred to common wheat from *Triticum ventricosum* and is localized in the short arm of chromosome 2A. This translocation also contains genes for resistance to stem (*Sr38*) and yellow (*Yr17*) rust, which are effective in Russia. The summary PCR results are presented in table 2 (table 2).

As a result of molecular screening, it was established that the Lr37 genes were identified in the List 25 cultivar; for the Myth variety - Lr10; for the Eltan variety - Lr10; Markola variety - Lr34; for the Malvina variety - Lr26; the variety Creator - Lr10; for the variety DB 1/05 - Lr10; the Evklid cultivar has the Lr10 gene; the variety Sumai aut - Lr34; in the Lebidka odes'ka variety - Lr34; Solara variety - Lr34; the variety Zhiva - Lr10, Lr34.

Table 2. Results of studying wheat varieties for the presence of Lr-genes using molecular markers

| Variety   | Lr9 | Lr10 | Lr19 | Lr20 | Lr24 | Lr34 | Lr37 | Lr41 | Lr47 |
|-----------|-----|------|------|------|------|------|------|------|------|
| List 25   |     | +    |      |      |      |      |      |      |      |
| Battum    |     |      |      |      |      |      |      |      |      |
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 following cultivars: Battum, Eltan, Evklid, Areal, Solara; the susceptible type of reaction (S) was noted in the Markola and Mallyska cultivars; medium susceptible type of reaction (MS) - in cultivars Lebidka odes'ka and Tvorets. [10,11,12]

The results obtained will be used in breeding programs for creating varieties resistant to leaf rust for conditions.

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