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Prospects of Using of Genetic Potential of *Triticum timopheevii* for Durable Defense of Common Wheat from Leaf Rust

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***Abstract*—Common wheat is the main grain crop in Russia. In the last decade, the development of leaf rust caused by the pathogenic fungus *Puccinia triticina* Erikss on the wheat was enhanced. To increase grain production and obtain environmentally friendly products, it is necessary to use varieties resistant to disease. Common wheat is cultivated over large areas in Western Siberia. In this region an aggressive Asian population of *P. triticina*, differed from the European one, exists on the crops. For durable defense of varieties from rust diseases, it is necessary to enrich the wheat genome with new resistance genes from the related cereals. The rare species *Triticum timopheevii* Zhuk. is considered a promising issue of resistance to fungal diseases, but its immunity has been confirmed mainly in the European part of Russia and in North America. The aim of the work was to monitor the resistance of *T. timopheevii* to leaf rust in Western Siberia and to determine the promising accessions for breeding programs. The adult resistance of *T. timopheevii* accessions was evaluated in the field conditions on intensive infectious background in 2011–2018 in the South of Western Siberia (Omsk). Additionally the reactions of seedlings to infection were estimated in laboratory. Damage of the plants by leaf rust was characterized by type, score/ percentage. The tendency to shift the type of seedling reaction from immune to susceptible was shown. Beside it the regular weak damage of adult plants of some *T. timopheevii* specimens was noted in the field in 2014–2018. Among a set of *T. timopheevii* accessions the promising specimens were determined with stable resistance to the Asian population of *P. triticina*, which are suitable for breeding programs. The work has begun to include the resistant accessions in the breeding process.**

Keywords*—*Common wheat, Triticum timopheevii, Puccinia triticina,* leaf *rust, Western Siberia.

I. Introduction

One of the promising directions to develop the Russian economy is the enhance of food production, including the wheat grains. Taking into account the increase in food standards in the world, the cultivation of environmentally friendly products is in demand.

The use of resistant varieties may decrease the use of chemical protection means against diseases, which leads to boost productivity and grain quality and is economically viable. However, large-scale cultivation of wheat, especially as a monoculture, creates the basis for the emergence of new races of pathogens, overcoming the resistance of varieties and triggers the development of epidemics [1]. To stabilize the phytopathological situation in an agrocenosis, the cultivation of heterogeneous varieties carrying different resistance genes is effective [2]. In connection with active race-forming processes in populations of wheat pathogens, it is necessary to search for effective issues of resistance to leaf rust into the related species and to involve them in the breeding programs

South of Western Siberia and the adjacent regions of Russia and the Republic of Kazakhstan are specialized in the production of common wheat with high grain quality. In this zone, the crop is grown on an area of several million hectares, making up the "wheat belt" [3]. Leaf rust caused by the pathogenic fungus *Puccinia triticina* Erikss. leads to significant losses of up to 25–30 % of the crop. In "wheat belt" in Siberia and Northern Kazakhstan an aggressive Asian population of *P. triticina* exists, which differs in virulence from the European one [4, 5]. In recent decades, rust epidemics in the zone were observed every 3–4 years [6]. In this regard, to protect varieties, it is necessary to use issues of resistance effective against different pathogen populations.

Tetraploid species *Triticum timopheevii* Zhuk. (A^bA^bGG) differs by genome from common wheat T. *aestivum* L. (BBA^uA^uDD) and belongs to a parallel evolution branch. The immunity of *T. timopheevii* to fungal diseases was first determined by N.I. Vavilov in the 1930s. In the twentieth century its immunity to leaf rust was confirmed in the European part of the Russia and in North America [7, 8, 9]. As the species *T. timopheevii* is immune to leaf rust in many regions of the world; it can be attributed as non-host species for *P. triticina*. *T. timopheevii* is considered a promising source of different genes, however, a limited set of resistance genes to leaf rust was transferred to wheat genome: *Lr18, Lr50, LrTt1* and *LrTt2* [10, 11]. To include new *T. timopheevi*i accessions in breeding programs, the information is needed on the stability of their resistance to the Asian population of leaf rust fungus.

The aim of the work was to monitor the resistance of *T. timopheevii* to leaf rust in Western Siberia and to determine promising accessions for breeding programs.

II. Material and methods

The objects of research were: the ten accessions of *T. timopheevii* from the Collection of the Federal Research Center N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). Nine accessions were native to Georgia and specimen k-47793 was created in the Department of Wheat Genetic Resources (VIR) by selection for disease resistance. Two botanical varieties were identified in the samples - *var. timopheevii* and *var. viticulosum*. The accessions k-30920, k-31684, k-35914, k-3591 included only plants of var. *timopheevii*, k-30922 – only *var. viticulosum*, the rest k-35915, k-38555, k-46956, k-47793, k-58666 – of different varieties. Spring common wheat cv. Pamyati Azieva was used as a susceptible control in laboratory and field experiments

To validate the results in 2011–2018 a two-stage investigations of the development of leaf rust on *T. timopheevii* specimens were conducted: 1) on seedlings in laboratory conditions; 2) on adult plants on the intense natural infectious background. Under laboratory conditions, the seedlings were infected with urediniospores of the Asian population of *P. triticina*, samples of which were collected annually in the field. For this purpose infected wheat leaves were gathered on commercial wheat crops. Later, in laboratory conditions, population samples were propagated on seedlings of susceptible variety of *T. aestivum*. In laboratory experiments the reaction type (score) to infection was determined on a scale: 0 - immunity, without pustules; 1-2 – resistance, pustules were surrounded by the necrotic zone; 3-4 – susceptibility, without necrosis [12]. The field experiments were carried out in the South of Western Siberia (Omsk). Since leaf rust in the region develops at the end of the growing season (late July – early August), the resistance of adult plants at stages from earing to wax ripeness was evaluated. A damage of the plants in the field was characterized by type, score / percentage [13].

III. Results

Taking into account the general trend of increasing harmfulness of rust diseases in the world, we conducted annual monitoring of leaf rust development on collection nursery in Western Siberia in 2011-2018. During the observation period (excluding arid 2012), there was an intensive disease development in the region, with the damage of susceptible bread wheat varieties reaching 70-100 % [14]. To test the stability of the resistance of *T. timopheevii* to leaf rust, the reaction type to infection at different stages of plant development was evaluated (the data 2011, 2014, and 2018).

Laboratory studies were carried out on large samples, in total, the reactions of 200 *T. timopheevii* plants (20 of each of specimens) were studied annually. In 2011, more than half of the plants were immune, and 46 % were resistant, on them only rare small pustules surrounded by necrotic zones were observed (type 1-2 score). Only single plants were susceptible; medium sized pustules surrounded by a zone of chlorosis formed on them (score 3) (Fig. 1, a). In 2014, a sharp shift in the proportion of resistant *T. timopheevii* plants was noted, while a significant part of them became susceptible (21%), a smaller part restored immunity. In 2018, a significant decrease of the proportion of immune (20 %) and susceptible plants (4 %) was mentioned, while the proportion of resistant increased up to 76 %. The development of urediniopustules on the leaves of the most susceptible accessions k-30920 and k-30922 is shown in Figure 2. Comparing the results of infection in 2011-2018, it should be noted that if in 2011 pustules (type 1–3 score) developed on 47 %, in 2018 – on 80 % of plants. This phenomenon suggests that within 8 years, the clones of the Asian population of *P. triticina* dramatically increased the ability to develop and reproduce on young plants of *T. timopheevii*.

To expand the wheat gene pool, it is important to use in plant breeding the species and specimens with stable resistance to the diseases. Therefore, data on reaction type of individual specimens were analyzed. Here presents the evaluation data on four samples of *T. timopheevii*, which showed the characteristic features of interaction with the Asian population of *P. triticina* (Fig. 1, b – d). When infecting seedlings, it was shown that the samples are represented by mixtures of plants with different resistance, i.e. populations. Using the k-30920 and k-30922 as an examples, it was demonstrated that in 2011–2018 overcoming immunity and an increase in the proportion of susceptible plants took place. At the same time, all plants of k-35915 and k-46956 retained immunity or high resistance to the disease.

Additionally, close attention was paid to the investigation of leaf rust development on the adult plants (stage of wax ripeness) on the intense natural infectious background in the field. In 2011, most of specimens were immune, only k-30922 demonstrated single microscopic pustules (1 score/ 5 %) (table). In 2014, pustules with type 2–3 score were detected on plants of k-30922, and in 2018 – of two specimens (k-30920, k-30922). On six other accessions the estimations varied from immunity to low damage (1-2 score / 5 %). At the same time, the specimens k-35915 and k-46956 showed immunity or high resistance for 8 years, which confirmed the of laboratory results. These samples are of interest as issues of resistance to leaf rust for plant breeding.

IV. Discussion

The resistance of non-host species is considered stable and, therefore, is of great interest for breeding programs from the view of durable protection of varieties to diseases [15]. However, sometimes the transition of previously non-adapted pathogenic fungi to new cultures has been registered. In particular, agent of rice blast *Magnaporthe oryzae* f. sp. *oryzae* overcame the resistance of non-host species: wheat, barley, and grasses in 1985. At present, this disease poses a threat to food security in different regions of the world [16].

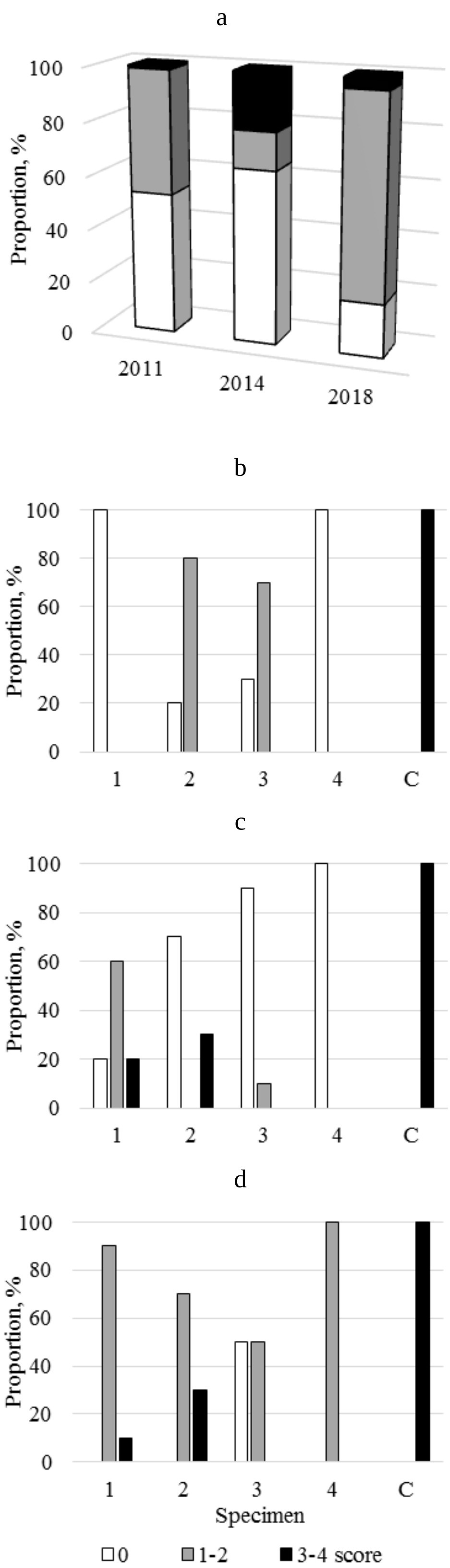

Fig. 1. Distribution of infected T. *timopheevii* plants according to reaction type at the seedling stage: a – results of the total assessment of 10 specimens; b, c, d – results of evaluation of different T. *timopheevii* specimens in 2011, 2014 and 2018, respectively. Accessions: 1 – k-30920, 2 – k-30922, 3 – k-35915, 4 – k-46956; C – cv. Pamyati Azieva, control. Score: 0 – immunity, 1-2 – resistance, 3-4 – susceptibility.

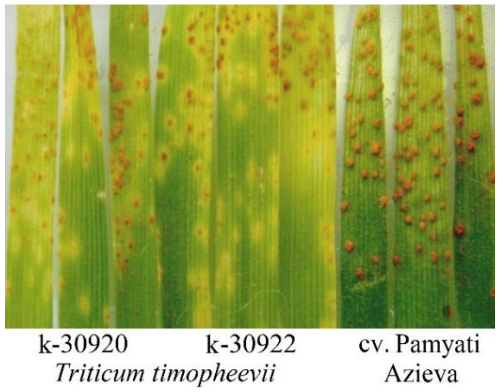

Fig. 2. Leaf rust on the seedling leaves of T. *timopheevii* specimens and susceptible common wheat cv. Pamyati Azieva.

TABLE I. RESULTS OF ESTIMATION OF LEAF RUST DEVELOPMENT ON ADULT PLANTS OF T. *TIMOPHEEVII* AND T. *AESTIVUM* IN THE FIELD

Specimen	Damage type, score/ percentage		
	2011 year	***2014 year***	***2018 year***
Pamyati Azieva – control	4/80	4/100	4/70
k-30920	0	0	1-2/10; 3/5
k -30922	0, 1/5	0; 2/5; 3/5	1-2/10; 3/5
k -35915	0	0	0
k -46956	0	0	0; 1/1

In the recent decade, an increase in the harmfulness of wheat leaf and stem rusts has been noted in Western Siberia [3, 14]. A previous study of the reactions of common wheat lines with known resistance genes introgressed from different related species showed that virulence for resistance genes that absent in the commercial wheat strains appeared in the West Siberian population of *P. triticina* [17]. In connection with the trend of enhancement of fungal diseases, it is necessary to monitor potential sources of resistance to rust diseases in the regions of mass cultivation of common wheat. The presented results indicate that accessions of *T. timopheevii* are polymorphic in reaction type, which confirms the previously obtained data on the heterogeneity of the samples [17]. These facts can be explained by the fact that *T. timopheevii* belongs to rare species; it was cultivated in a mixture with einkorn wheat in the form of landraces in Western Georgia [7]. In this regard, many of landraces represent populations consisting of forms differed in morphology, physiology and resistance to diseases

The presented data confirm the previously obtained information on the appearance in the Asian population of *P. triticina* virulent clones to some of *T. timopheevii* plants at seedling stage [5]. Current results demonstrate ongoing changes in the properties of *P. triticina* clones. The tendency of accumulation of clones capable to infect the juvenile plants of previously immune species is clearly visible from the results of the summarized evaluations of *T. timopheevii* specimens in 2011-2018. While in 2011 more than half of *T. timopheevii* plants were immune, i.e. prevented the appearance of pustules, in 2018 the rust fungus was able to reproduce on 80 % of the plants used in the experiments. When infected with *P. triticina* population collected in 2014, a sharp increase in the proportion of susceptible plants (more than 20 %) was noted. However, when infected by the 2018 population, the proportion of resistant plants (type 1-2 score) increased simultaneously with a decrease in the proportion of susceptible and immune plants. Such variations may be explained by the low viability of new clones virulent to *T. timopheevii* plants. According to Yu.T. Dyakov [1] a new virulent clone to survive in a population have to accumulate a set of modifying genes to increase its fitness and adaptation to the host and environmental conditions. The probability of accumulation of modifiers increases with the widespread cultivation of varieties protected by the corresponding gene, as well as under weather conditions suitable for epidemics. Since varieties protected by *T. timopheevii* genes have not yet been cultivated in Western Siberia, so far there is no basis for the selection and rapid fixing of the corresponding clones in Asian population of *P. triticina*.

It was previously shown that the immunity of *T. timopheevii* to leaf rust is determined by a set of defense mechanisms that suppress the development of pathogen on the leaf surface and in plant tissues [18]. The revealed changes in the ratio of immune, resistant, and susceptible plants in the specimens indicate that the pathogen gradually overcomes some of the protective barriers of the species *T. timopheevii*. The susceptibility of some *T. timopheevii* plants proves the appearance of clones interacting with them in a race-specific manner. Previously, overcoming of leaf rust resistance of introgressive wheat lines with single *T. timopheevii* genes was also shown in Western Siberia [19].

Currently, it is known that wheat resistance to rust diseases may be manifested at different development stages, in seedlings (seedling resistance) or adult plants (adult resistance). It is believed that adult resistance is nonspecific and can provide durable resistance of varieties [20]. In our experiments, the seedlings showed greater compatibility with the pathogen than adult plants. This may be explained by the significance of adult resistance in the *T. timopheevii* protection from leaf rust in field conditions. Among the genes introgressed from *T. timopheevii* to *T. aestivum*, only *Lr50* was indicated as an adult [10], with its low efficacy previously shown in Western Siberia [17]. However, in recent years even on adult plants of two specimens some fungal pustules with a different type were regularly revealed.

In conditions of increasing aggressiveness of the *P. triticina* population to *T. timopheevii* in Western Siberia in 2011-2018, two specimens k-31684 and k-35915 retained immunity or high resistance at the seedling stage. Moreover, in the k-35915, the proportion of immune plants even increased to 50 % in 2018. The same specimens showed high resistance to the disease in the field. Most likely, the accessions have additional genes / gene combinations that protect them from the Asian population of *P. triticina*. The accessions k-31684 and k-35915 were included in the breeding programs implemented in the Omsk State Agrarian University, and the first generations of hybrids resistant to leaf rust were obtained.

In general, a decrease in the proportion of immune and an increase in the percentage of susceptible plants of *T. timopheevii* at the seedling stage, as well as a weak damage of adult plants, indicate a steady trend in the accumulation of clones with new properties in the *P. triticina* population. The results indicating a coevolution of Asian population of *P. triticina* with the species *T. timopheevii* in Western Siberia. It should be taken into account when determining the strategy for wheat breeding with use of *T. timopheevii* genes.

V. Conclusion

1. The *T. timopheevii* accessions been included in experiments differed in resistance to leaf rust and were represented by populations polymorphic in resistance leaf rust.

2. It was registered the significant increase of damage to *T. timopheevii* plants by leaf rust at the seedling stage and regular weak symptoms on adult plants of some accessions in Western Siberia.

3. Two *T. timopheevii* accessions k-31684 and k-35915 retained stable high resistance to leaf rust at any stages of development. These specimens are promising issues of resistance to leaf rust for breeding programs.

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