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Evaluation of Leaf Rust Resistance Genes in Durum Wheat Varieties in Kazakhstan

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ABSTRACT

Durum wheat (*Triticum durum* Desf.) varieties are characterized by extensive amplitude of variability in leaf rust (*Puccinia recondita f.sp. tritici*) resistance. Models of all resistance groups from highly susceptible to utterly resistant are observed when different fungus pathotypes are used. Five leaf rust resistance genes (*Lr*1, *Lr*3ka, *Lr*19, *Lr*23, *Lr*25) have been postulated in durum wheat varieties by the method of phytopathological testing. An effective resistance gene *Lr*23 was detected in the genotype of the overseas durum wheat varieties Albatross, Cocorit 71, VZ-187 and in the domestic variety Nauryz 6. Highly effective gene *Lr*19 was identified in Valdur and Dur varieties as well as *Lr*25 in Lakota and Macoun. These varieties are valuable donors in wheat breeding for disease resistance. Major part of Kazakhstani durum wheat varieties possesses adult plant resistance genes. Kargala 303, Hordeiforme 94-94-13, E-605, E-622 and some other durum wheat varieties are of interest for breeding and genetic research as well as for commercial use.

Keywords: effective gene, isogenic line, pathotypes, *Puccinia recondita*, testing, *Triticum durum*

INTRODUCTION

The aim of the research was to detect wheat leaf rust (*P. recondita f.sp. tritici*) resistance genes by phytopathological testing (postulation) with use of the fungus pathotype with certain virulence.

At present over 50 genes controlling wheat leaf resistance are known (McIntosh *et al.* 1995). The range of leaf rust (*Lr*-) genes effect differs. Part of them determines resistance of seedlings to some races of the pathogen, the other genes work only in the stage of adult plants. There are also *Lr*-genes that are effective in all stages of plant growth to the most part of existing pathotypes of the fungus. Most part of *Lr*-genes was transferred to the soft wheat genome in different ways. The knowledge of the durum wheat in this respect is considerably less. In publications there is a lack of information on genetics of durum wheat varieties resistance to this pathogen. Due to this we conducted the research in identification of juvenile leaf rust resistance genes in durum wheat.

In Kazakhstan durum wheat is grown on the acreage of 200-300,000 ha in the northern, eastern and western regions. Eighteen durum wheat varieties are sown in the republic and large areas are under Bezenchukskaya 139, Damsinskaya 90, Kustanaiskaya 52, Omskiy Rubin and SID-88. As compared to soft wheat this crop is more resistant to fungal diseases, especially to leaf rust under conditions of Kazakhstan and Russia (Vasilchuk 2001; Koishibayev 2002).

There are different approaches and methods in identification of wheat resistance genes to diseases: phytopathological testing, hybridological analysis and molecular labeling. Method of phytopathological testing or resistance gene postulation allows supposing existence of resistance genes in varieties without hybridological analysis. In this procedure test-strains (pathotypes) of the fungal agents labeled with the known virulence are used (Person 1959; Loegering *et al.* 1971, 1974; Knott and Johnson 1981).

The researchers of many countries use this method to postulate juvenile resistance genes in wheat varieties. For instance, two additional recessive genes of the juvenile leaf rust resistance in Leeds variety of durum wheat were postulated by this technique (Statler 1973), afterwards one or two recessive *Lr*-genes were found in Ramsey, D561 and D6733 varieties (Rashid *et al.* 1976). Later on juvenile Lr-genes were identified in the genotype of 8 durum wheat varieties (Zhang and Knott 1990). CYMMIT researchers identified *Lr*-genes of juvenile resistance in 9 Mexican durum wheat varieties with use of the pathotype BBB/B and other widespread pathotypes of *P. recondita tritici* (Singh *et al.* 1993).

According to Russian researchers resistance genes postulation in wheat varieties is effective when there are test cultures of the pathogens with virulence genes that overcome variety resistance (Smirnova *et al.* 1991; Kovalenko *et al.* 2005). For instance, Kolomiyets *et al.* (2005) postulated 11 genes of leaf rust resistance (*Lr*1, *Lr*3, *Lr*2a, *Lr*10, *Lr*14b, *Lr*16, *Lr*19, *Lr*23, *Lr*25, *Lr*26, *Lr*32) in 64 varieties of spring and winter wheat.

So, phytopathological testing (resistance gene postulation) is a basic technique of rust resistance gene identification in wheat varieties. However this work should be carried out under strictly controlled conditions with use of fungal pathotypes of known virulence.

MATERIALS AND METHODS

Kazakhstani and Russian commercial and advanced durum wheat varieties as well as foreign wheat lines were used in the genetic study. Leaf rust clones isolated in the south-east of Kazakhstan and in the Northern Caucasus (Russia) as well as clones from the Microbial Collection (RIBSP, Republic of Kazakhstan) were used in the experiments as test-cultures.

The structure of the leaf rust population was determined according to the identification system of Long and Kolmer (1989) based on inoculation of isogenic Lr-lines with *P. recondita* spores that we had modified. According to this system the plant reaction is determined on 16 lines divided into 4 groups of four lines. The first group includes isogenic *Lr*-lines 1, 2a, 2c, 3; the second – 9, 16, 24, 26; the third group – 3ka, 11, 17 and 30. The fourth was the set of lines *Lr*19, *Lr*20, *Lr*25 and *Lr*29 (additional set for Kazakh-

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Table 1 Characteristics of the used wheat leaf rust pathotypes.

Pathotype	Origin	Virulence to *Lr*-lines
TKF/H	South-east of Kazakhstan	*Lr*1, 2a, 2c, 3, 16, 24, 26, 17, 30, 20, 29
SKF/G	South-east of Kazakhstan	*Lr*1, 2a, 2c, 16, 24, 26, 17, 30, 20
PHT/B	Northern Caucasus, Russia	*Lr*1, 2c, 3, 16, 26, 3ka, 11, 17, 30
THT/F	Northern Caucasus, Russia	*Lr*1, 2a, 2c, 3, 16, 26, 3ka, 11, 17, 30, 25, 29
KHT/C	Northern Caucasus, Russia	*Lr*2a, 2c, 3, 16, 26, 3ka, 11, 17, 30, 29

Table 2 Phytopathological testing of leaf rust resistance genes in durum wheat varieties.

Wheat varieties	Origin	Type of reaction to pathotypes, points					Resistance ratio* (%)		Postulated resistance gene
		Kazakhstani			Russian				
		TKF/H	SKF/G	PHT/B	THT/F	KHP/C	*R*	*S*	
Albatross	USA	; 1	0;	1 1+	2 3-	1+	80.0	20.0	*Lr*23
Bezenchukckaya 139	Russia	3+ 4	2+ 3	2+ 3	3+	2+	20.0	80.0	*Lr*1
Cocorit 71	Mexico	; 1	0;	1 1+	2 3- n	1 1+	80.0	20.0	*Lr*23
Dur	India	0	1+	2	0; 1	1+ 2	100.0	0.0	*Lr*19
Kargala 24	Kazakhstan	3+ 4	3	2+ 3	3+	2+	20.0	80.0	*Lr*1
Lakota	USA	0 ;	0	2+	3- 3	1 2	80.0	20.0	*Lr*25
Macoun	Canada	0 ;	0;	2+	3- 3	1 2	80.0	20.0	*Lr*25
Mondur	France	2 2+	1 1+	3 3+	3 3+	2+ 3	40.0	60.0	*Lr*3ka
Nauryz 6	Kazakhstan	; 1	0;	; 1+	2 3- n	1 1+	80.0	20.0	*Lr*23
Valdur	France	0	1+	2	0; 1	1+ 2	100.0	0.0	*Lr*19
VZ-187	Italy	; 1	0;	1 1+	2 3- n	1 1+	80.0	20.0	*Lr*23

* *R*: resistant, *S*: susceptible

stan). According to combination of responses of resistant (*R*) and susceptible (*S*) plants each rust agent isolate was coded in letters. As a result each pathotype has a code including 4 consonants of English alphabet from B through T.

The experiments were carried out in the greenhouse rooms with controlled conditions (temperature: +22…+24°C, illuminance: 10000-15000 lux, light period: 16 hours). Eight-ten-days seedlings of the varieties under study with unknown resistance genes and isogenic lines of Thatcher variety were inoculated with water suspension of the fungus uredospores. Five leaf rust pathotypes with 10-16 virulence genes were used in phytopathological assay (**Table 1**).

Plant resistance was evaluated in 12-15 days in the period of maximum disease manifestation in control (susceptible) plants. Affection type (in points) was evaluated according to the method of Mains and Jackson (1926) modified by Long and Kolmer (1989). Further on, comparison of reaction types of tested varieties and isogenic *Lr*-lines in the greenhouse as well as of field evaluation data allowed to make conclusions on presence or absence of resistance genes. The results of the analysis have also demonstrated dominance or recessiveness of the trait and resistance genes interaction. Moreover, resistance of the domestic and foreign durum wheat varieties to Kazakhstani and Russian leaf rust pathotypes was evaluated in the greenhouse.

RESULTS AND DISCUSSION

The study towards detection of juvenile leaf rust resistance genes by the method of phytopathological testing resulted in identification of five genes: *Lr*1, *Lr*3ka, *Lr*19, *Lr*23, *Lr*25 (**Table 2**).

One and the same gene of juvenile resistance *Lr*19 that is effective worldwide was postulated in wheat varieties Valdur (France) and Dur (India) on the basis of the phytopathological testing. Moreover, resistance gene *Lr*25 that is effective in Kazakhstan for many years has been detected in the tested durum wheat varieties (Rsaliyev Sh, Rsaliyev A 2008). The results of our research show that Russian pathotypes THT/F and KHP/C are highly virulent to the durum wheat varieties with *Lr*25 gene. According to published data recently in the Northern Caucasus and other regions of Russia the number of the pathogen clones virulent to *Lr*25 gene increased markedly (Plakhotnik and Kurbatova 2004; Mikhailova *et al.* 2002; Lind and Gultyaeva 2007). We postulated this gene in Lakota (USA) and Macoun (Canada) varieties.

According to Magomedov (1997) the most part of durum wheat varieties with leaf rust resistance in juvenile phase is protected by *Lr*23. The analysis of our findings has shown the presence of *Lr*23 in the genotype of the Mexican

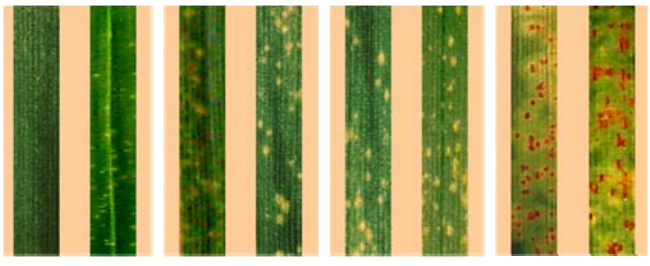

Fig. 1 Manifestation of leaf rust pathotypes in durum wheat varieties with *Lr*23 gene. (**A**) SKF/G, (**B**) TKF/H, (**C**) PHT/B and KHP/C, (**D**) THT/F.

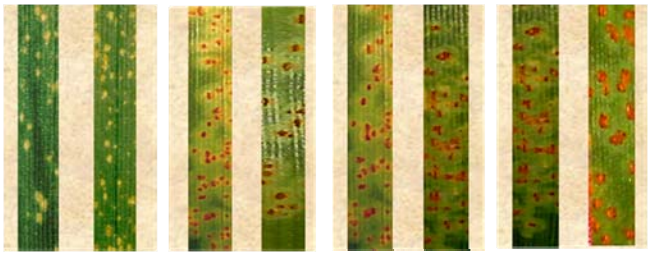

Fig. 2 Manifestation of leaf rust pathotypes in durum wheat varieties with *Lr*3ka gene. (**A**) SKF/G, (**B**) TKF/H, (**C**) KHP/C, (**D**) PHT/B and THT/F.

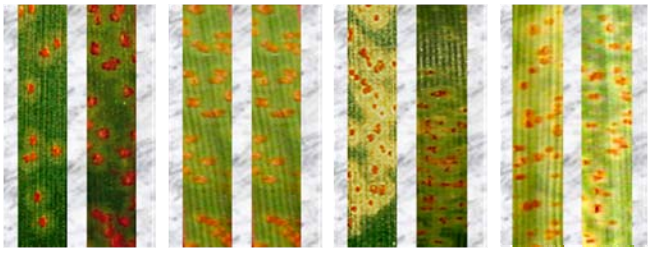

Fig. 3 Manifestation of leaf rust pathotypes in durum wheat varieties with *Lr*1 gene. (**A**) TKF/H, (**B**) THT/F, (**C**) SKF/G and PHT/B, (**D**) KHP/C.

Table 3 Immunological characterization of durum wheat varieties for resistance to leaf rust agent.

Variety	Origin	Type of reaction to pathotypes, points					Field resistance, leaf rust population*
		Kazakhstani		Russian			
		TKF/H	SKF/G	FHT/B	THT/F	KHP/C	
Acme	USA	1-	0	2+ 3	3+	2 2+	10*MR*
Aldura	USA	1+ 2	; 1-	0;	2 2+	2 2+	5*MR*
Altaiskaya niva	Russia	0	0;	2- 2	3+	0;	*R*
Altyn dala	Kazakhstan	2 2+	0;	3 3+	3	2+ 3	*R*
Crosby	USA	2	0 ;	0; 1-	2+ 3	2 2+	*R*
Damcinskaya 90	Kazakhstan	2+ 3	2-	0; 1+ 2-	2+ 3	2+	20*MS*
E-605	Kazakhstan	2 2+	2- 2	0; 1	2	2+	*R*
E-622	Kazakhstan	2 2+	2- 2	0; 1	2	2+	*R*
Hordeiforme 254	Kazakhstan	2 3-	; 1-	3 3+	3+ 4	3	*R*
Hordeiforme 426	Russia	3	; 1	0; 1	3	0;	*R*
Hordeiforme 429	Russia	4	0	3	3+	3+	5*MR*
Hordeiforme 94-94-1	Russia	1-	0	2+ 3	3+	2 2+	5*MR*
Hordeiforme 94-94-13	Russia	2-	0	2 2+	2+	2+ 3	5*MR*
Karabalykskaya chernokolosaya	Kazakhstan	3-	1- 1	3 3+	2+	3+ 4	*R*
Kargala 3	Kazakhstan	2-	1- 2	2+ 3	2+ 3	3-	5*MR*
Kargala 303	Kazakhstan	2-	2	2	2+	1+ 2	*R*
Kustanaiskaya 52	Kazakhstan	2 2+	0	3+ 4	3	3	5*MR*
Lan	Kazakhstan	2+ 3	2	0 0;	2+	3	*R*
Nauryz 2	Kazakhstan	3	2+ 3	3 3+	3+ 4	3+	20*MS*
Randur	France	1+ 2	0	0;	2-	2+ 3	*R*
Seymur	Kazakhstan	2 2+	2+ 3	2+ 3	3-	3	5*MR-MS*
SID-88	Kazakhstan	3 3+	3-	0; 2+	2+ 3	3+	10*MS*
Subastrale 489	Russia	2-	0;	3 3+	3+	2+	5*MR*
Svetlana	Russia	3	1	2+	2+ 3	2+	5*MR*
V.Z.-D	Italy	2- 2	1 2-	0;	2- 2	2	*R*
Wakooma	Canada	1+ 2	1+	1 2-	0; 2	2+	5*MR*

* *R*: resistant, *MR*: moderate resistant, *MS*: moderately susceptible (Roelfs *et al.* 1992)

durum wheat varieties Albatross and Cocorit 71, Italian variety VZ-187 and Kazakhstani variety Nauryz 6 (**Fig. 1**).

It should be noted that among used leaf rust clones pathotype THT/F was the most virulent to the varieties with *Lr*23 gene (**Fig. 1D**). Inoculation of tested varieties and *Lr*23 line with this pathotype results in high type of infection (3 points) with typical necrotic spots. Moreover, in the phase of adult plants all these varieties and isogenic *Lr*23 line demonstrated similar resistance to leaf rust. This is the evidence that in our region *Lr*23 gene is able to protect varieties against the disease during the entire period of plant ontogenesis.

Juvenile resistance gene *Lr*3ka was postulated in Mondur, French variety, developed with use of lines from Near East, North America and Italy. This variety and isogenic line *Lr*3ka demonstrated low type of infection (1+ and 2+ points) by pathotypes SKF/G and TKF/H (**Fig. 2A, 2B**), and high type of infection (3 and 3+ points) by pathotypes PHT/B, THT/F and KHP/C (**Fig. 2C, 2D**). It may be the result of the gene resistance dose effect when dominance of resistance to some pathotypes and recessiveness to another pathotypes is observed. Expressiveness of *Lr*3ka gene does not manifest itself in this variety since the gene has lost its effectiveness to Kazakhstani leaf rust population in all phases of plant growth.

The results of the phytopathological testing have shown that Bezenchukskaya 139 and advanced variety Kargala 24, widely used in the northern and southern regions of Kazakhstan, carry *Lr*1 juvenile resistance gene in their genotype (**Fig. 3**). However these varieties and the postulated gene show different types of infection in the field: *Lr*1 line is highly susceptible and the durum wheat varieties are resistant to the local leaf rust population. Perhaps it may be explained by the fact that Bezenchukskaya 139 and Kargala 24 have one or two more non-identified additional genes protecting the adult plants against the disease.

Lines E-605 and E-622 (RIBSP) demonstrated similar resistant types of infection by all used wheat leaf rust pathotypes. The identical data were obtained for Acme and Hordeiforme 94-9-1 with the same types of reaction to infection by some pathotypes. Judging on these data one may suppose that these lines posses the same gene(s) (**Table 3**). It should be noted that Kargala 303 in the phase of seedlings manifested resistant type of infection (1 and 2 points) by all used wheat leaf rust pathotypes. This case evidences the field or horizontal resistance of Kargala 303 variety to leaf rust.

Thus the results of the study show that durum wheat is characterized by wide range of variability in leaf rust resistance. When larger number of the fungus pathotypes is used all types of resistance from highly susceptible to absolutely resistant are observed.

CONCLUSIONS

Five leaf rust resistance genes (*Lr*1, *Lr*3ka, *Lr*19, *Lr*23, *Lr*25) were identified by the method of phytopathological testing in durum wheat varieties. Effective resistance gene *Lr*23 was postulated in the genotype of foreign durum wheat varieties Albatros, Cocorit 71 and VZ-187 and in variety Nauryz 6 (Kazakhstan). Highly effective gene *Lr*19 was detected in Valdur and Dur varieties, *Lr*25 in Lakota and Macoun varieties. These wheat varieties are valuable donors to be used in breeding for immunity.

Among the tested durum wheat varieties and lines Aldura, Altaiskaya niva, E-605, E-622, Hordeiforme 94-94-13, Kargala 303, Lan, V.Z.-D, Wakooma and so on are of great interest both for breeding-genetic studies and for commercial use in Kazakhstan.

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