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Analysis of the Virulence of Pathogens Populations Wheat (*Triticum aestivum* L.) Leaf Rust

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***Abstract* – Wheat leaf rust caused by *Puccinia recondita* Rob. ex Desm. f. sp. *Tritici* is a serious fungal wheat disease of global occurrence. This study carried out to analyze the determination of virulence was based on infection types on Thatcher near-isogenic lines (NIL) with the resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr3ka, Lr3bg, Lr9, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr19, Lr20, Lr21, Lr22a, Lr23, Lr24, Lr25, Lr26, Lr27+31,* Lr28, *Lr29, Lr30, Lr32, Lr33, Lr36, Lr37, Lr38, Lr39, Lr40, Lr41, Lr42, Lr43, Lr44, Lr45, Lr47, Lr48, Lr49, Lr50, LrB and LrW(52)* respectively. Thatcher with lines *Lr9, Lr19, Lr14b, Lr24, Lr25, Lr26, Lr28, Lr29, Lr41, Lr42, Lr43, Lr45, Lr47, Lr50* fungus pustules were absent or completely virulent in fungus except with line *Lr11*.**

***Keywords* – *Puccinia Recondita, Lr* genes, Resistance, Wheat.**

I. INTRODUCTION

Wheat (*Triticum aestivum L.*) is a dominant crop for human food and livestock feed. In Mongolian harsh climatic condition, it is economically necessary to introduce wheat varieties with stable characteristics of yield and quality. Leaf rust is a one of the most common wheat diseases in the main crop production areas of Mongolia. When conditions are favorable for fungus reduction in grain yield can reach 20-32%. The cultivation of resistant varieties is a key element of integrated protection of cultural phytopathogen to ensure the stability of agricultural production. The successful creation of varieties with durable resistance depends not only on the genetic diversity of the initial selection of material and its adaptability, but also on trends in intrapopulation structure of the agent. It is important to know the dynamics of virulence and aggressiveness of physiological races of the fungus to the cultivated varieties and donor stability, to timely identificate of new races and pathotypes, to maintain a constant search for the host resistance genes, effective against infection with artificial plants. This study was initiated to determine the virulence population of *P. recondita* in wheat in central agricultural zone in Mongolia.

II. MATERIALS AND METHODS

Collection of Wheat Leaf Rust Samples

Virulence in the population *P. recondita* in wheat (*Triticum aestivum* L.) was investigated at the Institute of Plant Protection of Mongolia (IPP) in 2011. For the study of population and sustainability of wheat in laboratory used a range of methods proposed by L.A.Mihaylovoi and K.V.Kvitko (1970). They methods are based on the solution of benzimidazole (40-60 ppm), which supports metabolism in segments of wheat leaves at the level, which type of response intervals for the type of reaction of intact plants. This type of plant response was taken into account in 7-10 days after inoculation. Breeding population and monopustul isolates were determined in tests of detached seedling leaves, maintained in Petri dish on cotton wool soaked in a solution of benzimidazole.

Inoculation of Wheat Leaf Rust Differential Hosts

Inoculation was carried out by applying the leaves collected in the field covered by rust pustules to the surface of the seedling leaves. After inoculation, the Petri dishes were placed on a day to diffuse light and then - in the brightness bench 2500-3000 l at a temperature of 22-24°C. For 7-8 days after the inoculation disputes handshaking collected into tubes. To clone the population after the development of pustules, gently holding the leaf segments with forceps cut the sheet with one segment of pustules and inoculate the leaves a group of segments in a cup. Formed in 7-8 days controversy shake off the tube and perform further multiplication to obtain a sufficient number of spores to analyze virulence. Total allocated 10 clones.

Phenotyping Differential Sets

To define the phenotype of clones on the ground of virulence (race) used following procedures. Segments of leaves Lr-wheat lines 7 -10 days of age about 3-5 cm long laid out in a cell on the glass, wrapped with filter paper, the ends of which are omitted in the solution of benzimidazole, poured on the bottom of the cell. The ends of the segments sheltered cotton swab moistened with benzimidazole. Lr-inoculation lines held spraying a spore suspension of the population, as well as 10 individual clones. After inoculation of the cell covered glass for 10-15 h were kept in diffuse light, and then transferred to bench fixed temperature 20-21°C.

Determining the type of infection when inoculated pathogen populations and clones 7-10 days after inoculation on a scale of Mines and Jackson (Table 1).

Table 1. The main classes of types of infection wheat leaf rust

Type of infection	Type of infection	Symptoms
0	Immunity	No visible symptoms;
;	High resistance	Hypersensitive spot;
1	Resistance	Small pustules with necrosis;
	Average resistance	Small, medium-sized pustules surrounded by necrosis or chlorosis
3	The average sensitivity	Medium-sized pustuleswithout chlorosis;
4	Sensitivity	Large pustules without chlorosis;
X(1,2)	Stability	pustules of different types
Y	Sensitivity	pustules of various sizes, with the size of the top of the list
Z	Sensitivity	pustules of various sizes, with the size of the base plate

Typically, the type of infection, which manifests itself in intact plants (seedlings), coincides with the type of response to the separated leaves, stored in a solution of benzimidazole. Note, however, that in the latter case there may be some difficulties with the classification of types of "2", "2 -", "2 +", because it stretches to benzimidazoles is slightly higher than in intact plants. However, the type of infection is 0, 1, 3, 4 usually coincides with the type of infection, and results in the intact plants.

Designation of Races

Designation phenotypes fungus: Virulence monopustul isolates of the fungus were tested for 24 TsLr-lines. The first four lines of resistance genes involved in the international recruitment of differentiators (group 1: *Lr1, Lr2a, Lr2c, Lr3*; group 2: *Lr9, Lr16, Lr24, Lr26*; group 3: *Lr3ka, Lr11, Lr17, Lr30*; group 4: *Lr10, Lr18, Lr14a, LrB*) (Long, Kolmer, 1989), the following two groups of lines were supplemented with genes informative for differentiation of P. triticina in Europe and Russia (group 5: *Lr2b, Lr3bg, Lr14b, Lr15;* group *6: Lr19, Lr20, Lr21, Lr28*). Decoding letter code used to identify phenotypes is shown in Table 2.

Table 2. Phenotypes code P. recondita

	group 1: *Lr1, Lr2a, Lr2c, Lr3* group2: *Lr9, Lr16, Lr24, Lr26* group 3: *Lr3ka, Lr11, Lr17, Lr30* group 4: *Lr10, Lr18, Lr14a, LrB* group 5: *Lr2b, Lr3bg, Lr14b, Lr15* group 6: *Lr19, Lr20, Lr21, Lr28*			
B	L	L	L	L
C	L	L	L	H
D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

L - low infection type (avirulent)
H - high type of infection (virulence).

Calculating indexes intrapopulation diversity and differences between populations in virulence and DNA was performed using the software package Virulence Analysis Tool (VAT) (Kosman et al., 2008).

III. RESULTS

To identify highly effective Lr-resistance genes evaluated the full set of control lines Lr-(Lr1-Lr50) (Table 3), infection of the Mongolian population and monopustul isolates. Revealed that on the line with the genes Lr9, Lr19, Lr14b, Lr24, Lr25, Lr26, Lr28, Lr29, Lr41, Lr42, Lr43, Lr45, Lr47, Lr50 fungus pustules were absent, and.

respectively, virulence to them was 0%. In the Line of Lr11 pustules when inoculated population was significantly less compared to susceptible lines. Analysis of the clones revealed that most of them (70%) were avirulent on the line with this gene. Type of reaction to the line with a gene Lr44 was receptive, but the pustules were smaller in size compared to other lines.

Table 3. The virulence of the population of Bornuur of Mongolia to the lines of Thatcher with juvenile resistance genes (%)

Lr-lines	Type of reaction											Percent of virulent isolates
	population	K1*	K2	K3	K4	K5	K6	K7	K8	K9	K10	
Lr1	3	3	3	3	3	3	3	3	3	3	3	100
Lr2a	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr2b	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr2c	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr3a	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr3bg	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr3ka	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr9	0	0	0	0	0	0	0	0	0	0	0	0
Lr10	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr11	3**	3	0	0	0	0	0	0	3	3	0	30
Lr14a	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr14b	0	0	0	0	0	0	0	0	0	0	0	0
Lr15	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr16	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr17	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr18	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr19	0	0	0	0	0	0	0	0	0	0	0	0
Lr20	3	3	3	3	3	3	3	3	3	3	3	100
Lr21	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr22a	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr23	3	3	3	3	3	3	3	3	3	3	3	100
Lr24	0	0	0	0	0	0	0	0	0	0	0	0
Lr25	0	0	0	0	0	0	0	0	0	0	0	0
Lr26	0	0	0	0	0	0	0	0	0	0	0	0
Lr27+31	3	3	3	3	3	3	3	3	3	3	3	100
Lr28	0	0	0	0	0	0	0	0	0	0	0	0
Lr29	0	0	0	0	0	0	0	0	0	0	0	0
Lr30	3	3	3	3	3	3	3	3	3	3	3	100
Lr32	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr33	3	3	3	3	3	3	3	3	3	3	3	100
Lr36	0	0	0	0	0	0	0	0	0	0	0	0
Lr37	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr38	0	0	0	0	0	0	0	0	0	0	0	0
Lr39	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr40	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr41	0	0	0	0	0	0	0	0	0	0	0	0
Lr42	0	0	0	0	0	0	0	0	0	0	0	0
Lr43	0	0	0	0	0	0	0	0	0	0	0	0
Lr44	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	100
Lr45	0	0	0	0	0	0	0	0	0	0	0	0
Lr47	0	0	0	0	0	0	0	0	0	0	0	0
Lr48	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
Lr49	0	0	0	0	0	0	0	0	0	0	0	0
Lr50	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0
LrB	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100
LrW(52)	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	3-4	100

* K1 - clone 1, etc.
3 ** - number of pustules is much less than in other susceptible lines
3 - type receptive, but less than 3.

Racial composition defined by the North American nomenclature is shown in Table. 4. A total of two race, which differed only in virulence to the gene Lr11 (Table 4).

Table 4. The racial composition of the Bornuur population

Phenotype (race)	Frequency of occurrence,%
TGPTRJ	70
TGTTRJ	30

To assess intra-phenotypic diversity in the composition of the index used Nei's Hs (Nei, 1973), describing the heterogeneity only on virulence frequencies, normalized Shannon index Sh (Shannon, Weaver, 1949; Kolmer et al., 2003), with different types of populations for phenotypic composition and index Cosman KWm (Kosman, 1996; Kosman, Leonard, 2007), estimates the total population variability in virulence and phenotypic composition. According to all the statistical indices for all study populations obtained equivalent results (low diversity). Thus, the use in the analysis of non-representative of infectious material (only one population of which includes a limited number of clones) resulted in low genetic diversity intrapopulation Mongolian population (Table 5.6).

Table 5. Intrapopulation diversity indices in the Bornuur population

Index	Value
Hs	0.018
Sh	0.265
KWm	0.025

Table. 6. Comparison of clones with each other: the degree of difference

Clones	2	3	4	5	6	7	10	1	8	9
2	0									
3	0	0								
4	0	0	0							
5	0	0	0	0						
6	0	0	0	0	0					
7	0	0	0	0	0	0				
10	0	0	0	0	0	0	0			
1	0,042	0,042	0,042	0,042	0,042	0,042	0,042	0		
8	0,042	0,042	0,042	0,042	0,042	0,042	0,042	0	0	
9	0,042	0,042	0,042	0,042	0,042	0,042	0,042	0	0	0

IV. Conclusion

Ten isolates of *P. recondita* collected in Tov province were tested for virulence to plants of Thatcher isogenic wheat lines with leaf rust resistance genes *Lr1-50* combined. All of the isolates had low infection type to the Thatcher lines with *Lr9, Lr19, Lr14b, Lr24, Lr25, Lr26, Lr28, Lr41, Lr42, Lr43, Lr45, Lr47* and *Lr50* produced very low or intermediate avirulent infection type. All isolates had lower infection type and lower rust severity on the Thatcher line with *Lr11* compared with Thatcher. The isolates were polymorphic for virulence to the Thatcher line with *Lr44*. Other isolates produced virulent infection type.

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