POSTULATION OF LEAF RUST RESISTANCE GENES IN CULTIVARS OF SOFT WINTER WHEAT

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Abstract

Leaf rust caused by fungi Puccinia triticina Rob. ex Desm. f. sp. tritici Erikss. et Henn., is a major disease affecting cultivars of soft winter wheat (Triticum aestivum L.), grown in the South of Russia. The objective of this study was to characterize seedling leaf rust resistance present in 12 cultivars of soft winter wheat. Leaf rust infection types (ITs) produced on cultivars by 16 isolates were compared with ITs produced on a standard set of Tatcher near-isogenic lines. Seedling resistance genes Lr: 1+, 2a, 3ka, 15, 16, 23, 33, 34 were postulated in 8 cultivars. The gene Lr23 occurred more often than the other ones. None of these genes, except for Lr23, in adult plants is effective for the population of leaf rust in the South of Russia. Adult plants leaf rust resistance for cultivars and lines was assessed in filed conditions on experimental fields of All-Russian Research Institute of Biological Plant Protection in 2014.

Key words: *soft winter wheat, resistance genes, leaf rust, postulation*

Leaf rust, caused by fungus Puccinia *triticina* Rob. ex Desm. *f. sp. tritici* Erikss. et Henn., is the most widespread and regularly occurring wheat disease in the South of Russia. Wheat cultivars cultivated in the region require leaf rust resistance since the pathogen may occur from early seedling stage up to harvesting time. With moderate plant affection grain yield decreases by 10%, under conditions of severe epiphytotic the decrease may reach 50-70 % (Lebedev et al., 1994). When plants are affected during earing stage by 80-100 % grain losses are 35-50 % respectively, and during flowering stage with the same degree of damage - 26-35% (Tanskiy et al., 1998). Wheat cultivars with high level of race specific resistance to *P. triticina*, will eventually select the virulent races of the pathogen, which results into loss of resistance in these cultivars. Selection of virulent races may occur actively in the South of Russia, since leaf rust has the potential to overwinter successfully in this area.

Relatively few genetic studies of leaf rust resistance have been conducted with soft winter wheat sown in the South of Russia (Anpilogova et al., 2011; Volkova, Vaganova, 2015). The object of this study was to identify resistance genes *Lr* in 12 winter wheat cultivars released in the South of Russia, using phytopathological testing. The approach described in this study is based on Flor's law – «gene-for-gene» and has been used to quickly determine set of seedling leaf rust resistance genes in a group of soft winter wheat cultivars (Flor, 1971; Person, 1959). High and low ITs produced by virulently diverse group of pathogen under study were compared with ITs produced on a standard set of Tatcher near-isogenic lines. Isolates producing low ITs on specific *Lr* –genes in Tatcher differentiator lines, will also show the same behavior patterns to the cultivars carrying the same resistance genes. When more than one combination of resistance genes could give the similar reaction (have the same resistance phenotype), combination with a less number of resistance genes was used for describing the phenotype. This method was used for postulation of seedling resistance genes in CIMMYT spring wheats (Singh, 1993), hard red wheats (McVey and Long, 1993) and European winter wheats (Singh, 2001). In this study the postulate method was used for identifying probable genes providing seedling leaf rust resistance in 12 soft winter wheat cultivars cultivated in the South of Russia. Assessment of field resistance of the cultivars was conducted on experimental fields of All-Russian Research Institute of Biological Plant Protection in 2014, compared with Tatcher lines having seedling and adult plant leaf rust resistance to determine the presence of resistance genes.

MATERIALS AND METHODS

Seeds of 12 soft winter wheat cultivars (Pervitsa, Sila, Yubileynaya 100, Delta, Laska, Patriarch, Krasnodarskaya 99, Batko, Irishka, Lebed, Verta, Krasota) were obtained from the largest breeding center in the South of Russia – Krasnodar Agricultural Research Institute named after P.P. Lukiyanenko. Plants of each cultivar were grown in 50 ml planters filled with sand, on hydroponics using Knop nutrient solution (Smirnova, Alekseeva, 1988) in the greenhouse with a temperature of boxes from 18 to 25^0C with 16 hours period of lighting. 5 seeds of each cultivar were sown in each planter. 41 Tatcher differentiator lines were planted separately.

For postulating *Lr* genes 16 fungi isolates were selected, the races dominating on the South of Russia and races with low and high infection type (ITs) (Table 1). *P.trticina* isolates were assigned four-letter race designations based on high and low IT to 16 Thatcher isogenic line according to North American nomenclature (Long, Kolmer, 1989). 7-9-days-old seedling of 12 cultivars and 41 lines were separately inoculated with spores suspension of each fungi isolate (with sprayer) and placed into humidity chamber with a temperature 20^0C for 16 hours. Then the inoculated plants were returned in greenhouse conditions. The assessment was conducted on the 12^{th} day after inoculation using scale in Long and Kolmer 1989): «0 - no hypersensitive flecks, necrosis, or uredinia—0; - faint hypersensitive flecks; - distinct hypersensitive flecks; 1 - small uredinia surrounded by distinct necrosis; 2 - small uredinia surrounded by distinct chlorosis; 3 - moderate size uredinia without chlorosis or necrosis; 4 - very large uredinia without chlorosis or necrosis. Mixtures of two or more IT were recorded as ITs with the most common IT listed first» (Phytopatology 79:525-529). Designationsof + and – were added as superscripts to the 0t o 4 IT to indicate larger and smaller uredinia than normal, respectively. IT 0 to 2+ were considered as low, and IT 3 to 4 were considered as high.

The presence of juvenile leaf rust resistance genes in the soft winter wheat cultivars was postulated on the basis of comparing low and high IT to the infection types on the Thatcher isogenic lines (Table 1).

Table 1. Seedlings reaction of Tatcher isogenic lines (*Lr)* on 16 virulence phenotypes of *P.trticina*

Line with *Lr* gene	PGTS	KHRQ	KHKS	FHTS	KHRS	FVTS	KHRS	KHRQ	CGTS	THTS	THTS	TBTS	KHTS	TGTS	THTJ	THTS	Field severity, %
1	3	1	1	1	0;	0;1	0;	0;1	1	3	3	3	1	3	3	3	60MS
2a	2	3	3	2	3	2	3	3	2	3	3	3	3	3	3	3	50MS
2c	3	3	4	3	4	3	3	3	1	3	3	3	3	3	3	3	90S
3	4	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	90S
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0;	0	0
16	3	3	3	4	3	3	3	3	3	3	3	2	3	3	3-	3-	50S
24	0;	0;	0;1	0;1	0	3	0;1	0	0	0	0	1	0	0;1	0	0;1	5R
26	0;	3	4	3	3-	0	3	3	0	3	4	2	3	0	3	3	60MS
3ka	3	3	0	3	3	3	3	3	3	3	4	3	3	3	3	3	90S
11	3	3	3	3	4	3	4	4	3	4	4	3	3	3	3	3	80S
17	3	2	3	3	1	3	2	1+	3	3	3	3	3	4	3	3	10R
30	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	50S
B	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	90S
10	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	100S
14a	3	2	3	3	3	3	3	1,1+	3	3	3	3	3	3	3	3	80S
18	0	0	0	0;	0	0	0	0;1	0	0	0;1	0	0	0	0	0	10R

3bg	3	2-	4	3	1	3	3+	3	3	3	3	3	3	3	3	3	80S
14 b	3-	3	3	3	3	3	4	3	3	3	3	3	3	3	1+	3	50MS
15	1	1	0;	1	1	1	2	1	0	0;1	1	1	2	0;	3	2	30MR
19	0;	0	0	0;	0	0	0	0	0	0	0	0	0	0	1	3	5R
20	0;	0;	1	0	0	0;	0;	0;	0	0;	0	0;1	1	0	3	1	30MS
21	1	2	1	2	1	3	2	2	1	2	2	1	2	1	1	1	40MS
23	3	3	3	3	3	3-	3	3	0	3	3	3-	3	3	2	3	20MR
25	3	1	1	1	0;	0	1	1	0;1	1	3	3	1	0;	1	1	20MR
28	1	1	0	1	2	0	1	1	0	3	1	1	1	1	3	0;	15MR
29	1	0;	0	0;1	0;	0;	1	1	0	1	1	1	0;1	1	1	1	1R
32	1	1	1	1,1+	1	1	1	1	1	1	1	1	0;1	1	2	1	20MR
33	3	2	3	3	3	3	3	3	2	3	3	3	3	3-	2-	3	50S
34	2	3-	2	3	3	3-	3	3	3	3	2	3	3	1	3	3	60MS
36	3	2	3	3	1	3-	2	1	3	3	3	3	3	3-	1	3	10R
37	3	3	3	3	3	3	3	3	3	3	3	3	3	3-	3	3	10R
38	0;	0;	0;1	0;	0;	0	0;	0	0;	0;1	0;	1	1	0;	0;1	0;1	20MR
40	3	2+	3	3	1	3	3	2	3	3	3	3	3	3	3	4	30MR
41	0;	0	0;	0;	0;	0;	0	0	0	0;	0	0	0;	0	1	0;	5R
42	0;1	0	0	1	0	1	1	1	0	1	0	1	1	0;	1	1	0
43+24	0	0	0	0;	0;	0	0	0;1	0	0;	0	0;	0;	0	0;1	0	0
44	;1	1	3-	2	1	1	1	1	1	1+	2	1	1	0;1	2	1	10R
45	1	3	1	1	0;	1	1	1	3-	1	3	1	1	1	1	2	5R
47	0;	0;	0	0	0	0;	0;	0	0	0;	0	0;	0;	0;	0;	1	1R
Ech	3	3	3	3	3	3	3	3	3-	3	3	3	3	3	3	3-	60MS
W(52)	1	1+	2	1	1	1		11+	0	2	2	2	1	0;1	1	2-	10R

Wheat cultivars and lines were planted in the field in 6-row plots, 1 meter in length. The distance between plots was 0.5 m. Each plot had 50-60 plants. Infection was carried out with a natural population of the fungi. Severity degree and reaction type were recorded on May 25, when susceptibility of cultivar Tatcher had reached 70-90 %. The following evaluation scale was used: 0 – no flecks or uredinia; TR – non-significant number of uredinia; R – small uredinia with necrosis; M – mixture small and large uredinia with chlorosis; MR – moderate size uredinia with necrosis; MS- moderate size uredinia with chlorosis; S- large uredinia.

RESULTS AND DISCUSSION

Seedlings resistance

Infection types of 16 *P.trticina isolates*, used for studying 12 soft winter wheat cultivars are presented in Table 2.

Table 2. Seedlings reaction of 12 winter wheat cultivars inoculated with 16 virulent phenotypes of *P.trticina*

Cultivars	Postulated *Lr*-gene	PGTS	KHRQ	KHKS	FHTS	KHRS	FVTS	KHRS	KHRQ	CGTS	THTS	THTS*	TBTS*	KHTS	TGTS	THTJ	THTS*
Pervitsa	15	0	0;	1	0	0	0	0	0;	0	0;	0	0	0;	0;	3	0
Sila	3ka	3	3	0,2	3	3	3	3+	3	3	3	3	4	3	3	4	3
Yubileynaya 100	1+	3	0;	0	0	0	3	0	0;	2	0;	3	3	3	3	3	3
Delta	23	3	3	3	3	3	3	4	3	3	3	1	3	3	3	1	0
Laska	33, 34	1	3	1+	3	3	3	3	3	2	3-	3	3+	3	1	1	3
Patriarch	34+	3	3	3	4	3+	3	3	3	3	3	0;	4	3	3	4	4
Krasnodarskaya 99	34	3	3	3	3	3	4	3	3	3	4	1	3	3	3	2	4
Batko	34, 2a	3	3	3	2	3	2	4	3	3	3-	1	3	3	3	2	3
Irishka	?	0	0;	0;	1	0	0	0;1	0	0	0;	0;1	0	0;	0	0;	0
Lebed	?	1	0;	0	0	0	0;	0	0;	0	0	1	1	0	1	1	1
Verta	?	0	0;	1	1	0	1	0	0;	0	1	0;1	0	1	0	0;	0
Krasota	?	0	1	1	1	0;	0	1	0;	0;	1	0;	0	1	0	1	0;

*Isolates of THTS phenotype (4/61, 4/62, 5/39) differ by reaction on a full set of testers

Cultivar Pervitsa is characterized by high IT of 3 to THTJ isolate and a low IT (0, 0; 1) to all other isolates, which are avirulent to *Lr15*. Pervitsa is likely to have *Lr15* gene.

Cultivar Sila has low IT (0, 2) to KHKS isolate and high IT (3,3+,4) to all studied isolates, which shows that Sila is probably carrying *Lr3ka* gene.

Cultivar Yubileynaya 100 has low IT (0, 0; 2) to KHRQ, KHKS, FHTS, KHRS, KHRQ, CGTS, THTS isolates and high IT of 3 to all other isolates. KHRQ, KHKS, FHTS, KHRS, KHRQ, CGTS, THTS are avirulent to *Lr1*, and PGTS, THTS, TBTS, TGTS, THTJ are virulent to *Lr1*, which allows suggesting presence of *Lr1* gene in variety Yubileynaya 100.

Cultivar Delta is characterized by low infection type – from 0 to 1 to THTS, THTJ isolates and high IT of 3, 4 – to all other isolates. THTJ isolate is avirulent to *Lr23* gene. Cultivar Delta is probably carrying Lr23 resistance gene.

Cultivar Laska has low IT of 1, 1+, 2 to PGTS, KHKS, CGTS, TGTS, THTJ isolates and high IT of 3-, 3, 3+ to all other isolates. THTJ isolate is avirulent to *Lr33* gene, and PGTS, KHKS, CGTS, TGTS – to *Lr34* gene. Cultivar Laska is probably carrying *Lr23* and *Lr34* resistance genes.

Cultivar Patriarch is characterized by high IT of 3-, 3, 4 to all studied isolates except for THTS isolate. Isolate THTS is avirulent to *Lr34* gene, which allows suggesting presence of *Lr34* gene in cultivar Patriarch.

Cultivar Krasnodarskaya 99 has low IT of 1, 2 to THTS and THTJ isolates and high IT of 3, 4 to all other isolates. THTS isolate is avirulent to *Lr34* gene, which allows suggestin presence of *Lr34* gene in cultivar Krasnodarskaya 99.

Cultivar Batko is characterized by low IT of 1, 2 to four isolates: FHTS, FVTS, THTS, THTJ and high IT of 3-, 3, 4 to all other isolates. FHTS, FVTS isolates are avirulent to *Lr2a,* and THTS is avirulent to *Lr34* gene. Cultivar Batko probably has *Lr2a* and *Lr34* genes.

To four cultivars with high resistance: Irishka, Verta, Lebed, Krasota, differentiating fungi isolates were not found. It can be suggested that these cultivars carry *Lr* genes of *41, 42, 43+24, 47* type, which are effective against *P.trticina* in the South of Russia.

Field resistance

Cultivar Pervitsa (*Lr15*) has shown a moderate resistance and had severity degree of 15MR (moderate size uredinia with necrosis) (Table 3), line with *Lr15* gene - 30 MR.

Table 3. Leaf rust severity and resistance response of 12 winter wheat cultivars in the South of Russia in 2014

Cultivars	*Lr*-genes postulated	Field severity, %
Pervitsa	15	15 MR
Sila	3ka	15MS
Yubileynaya 100	1+	50MS
Delta	23	10R, MR
Laska	33, 34	30MS
Patriarch	34+	20M
Krasnodarskaya 99	34	60MS
Batko	34, 2a	40MS
Irishka	?	1R
Lebed	?	1R
Verta	?	5R
Krasota	?	5R

Cultivars Sila (*Lr3ka*), Yubileynaya 100 (*Lr1+*), Laska (*Lr33, 34*), Patriarch (*Lr34*), Krasnodarskaya 99 (*Lr34*), Batko (*Lr34, 2a*) had various leaf rust severity from 15MS to 60 MS (moderate size uredinia with chlorosis). Line Tatcher with *Lr3ka* gene had leaf rust severity of 90S, that with *Lr1* gene - 60MS, *Lr33* gene - 50S, *Lr34* gene - 60MS, *Lr2a*- 50MS. The obtained results indicate that field resistance provided by each mentioned gene separately is insignificant or nonexistent.

Cultivar Delta (*Lr23*) had shown moderate adult plant resistance and had severity degree of 10R (small uredinia with necrosis), MR (moderate size uredinia with necrosis). Line Tatcher with *Lr23* gene had the similar severity degree - 20MR.

In cultivars Irishka, Lebed, Verta, Krasota seedling resistance genes were not detected. In field conditions these samples had shown high resistance and had low leaf rust severity from 1R to 5R (small uredinia with necrosis). These cultivars are likely to have effective combination of seedling and adult plant resistance genes.

CONCLUSION

On the basis of leaf rust infection types of studied 12 cultivars of soft winter wheat cultivated in the South of Russia, genes f\in eight cultivars were postulated. Seven *Lr* genes were detected*: 1+, 2a, 3ka, 15, 23, 33, 34*. No mentioned genes except for Lr23, provide adult plant leaf rust resistance in the South of Russia. Most commonly occurring was *Lr34* gene, carried by the ancestor – cultivar Bezostaya 1. Presence of *Lr34* gene provides non-specific leaf rust resistance. That's why wheat cultivars carrying only this resistance gene may later become susceptible to the disease due to increasing epidemic load. Ability of *Lr34* gene to interact with other genes for providing high leaf rust resistance is known, which is the important factor for breeding programs. Cultivars with postulated effective genes of resistance to *P.trticina*, are recommended for use in breeding and in territorial distribution in the South of Russia.

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