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PHYSIOLOGICAL SPECIALIZATION OF WHEAT LEAF RUST (*Puccinia triticina* Eriks.) IN BULGARIA

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ABSTRACT

Leaf rust on wheat caused by *Puccinia triticina* Eriks. is a disease widespread in all parts of the world where wheat is a main cereal crop. The race, pathotype and genetic variability of the pathogen's population in Bulgaria were investigated. The virulence of the pathogen population was studied on *Thatcher* near isogenic lines with genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr9, Lr11, Lr15, Lr17, Lr19, Lr21, Lr23, Lr24, Lr26* and *Lr28.* A total of 221 isolates were analyzed. Seventy-one phenotypically different pathotypes were identified. Pathotypes 63562, 63573 and 63762 had the highest frequency of occurrence. For the first time in this population 25 entirely new pathotypes were identified. The genetic variability was represented by 86 genetic formulae for virulence. The most common combination was 1, 9, 15, 19, 24, 28 / 2a, 2b, 2c, 3, 11, 17, 21, 23, 26 (37.9%). The genes for resistance demonstrated variable efficiency. During this period the immunity was conferred by the following genes: *Lr22a, Lr22b* and *Lr43*. Genes *Lr1, Lr3ka, Lr9, Lr19, Lr25, Lr28, Lr40, Lr41, Lr42, Lr45, Lr47* and *Lr50* demonstrated very good to high efficiency. During the investigated period, pathotypes were found which were overcoming the resistance of the two strong genes *Lr9* and *Lr19*.

Keywords: *P. triticina,* pathotypes, virulence, effectiveness, *Lr* genes.

INTRODUCTION

Common wheat *T. aestivum* is a main cereal crop worldwide. Between 10 and 15 million ha are annually sown with this crop in Bulgaria. Obtaining of high and stable yields from wheat is conditioned by the cultivar's productivity, the varietal structure, the applied agronomy practices and the protection against diseases and pests. One of the most widespread and harmful diseases on wheat, which is the main limiting factor in the production of commercial varieties, is brown rust caused by *Puccinia triticina* Eriks. The monitoring of the pathogen population is important for each breeding program. The analysis of the race and genetic specialization dynamics of the pathogen and the investigation of its virulence provide data on the pathotypes dominant in the population and on the identification and migration of new virulent races. In Bulgaria, a large number of pathotypes of different virulence are annually identified, the investigations in this direction are being presented in a series of publications: (Todorova, 1999; Todorova and Kiryakova, 2000, 2001; Karzhin et al., 2003; Stefcheva and Maneva, 2006; Kiryakova, 2007; Ivanova, 2012, 2014). Such investigations in Bulgaria date back to 1930 (Daskalov, 1930). The data from these researches over the years reveal that *Puccinia triticina* is characterized by high race and genetic diversity and that its race composition is not constant but highly variable. Such investigations are carried world-wide, in all regions where wheat is a main crop - Kolmer (1999), Kolmer et al. (2012) in the USA and Canada, Park et al. (2002) in Australia, Liu and Chen (2012) in China, Goyeau et al. (2006) in France, Mantovani et al. (2011) in Italy, Hanzalova and Bartos (2006), Hanzalova et al. (2008, 2012) in the Czech Republic.

The aim of this investigation was to study the pathotypic, racial and genetic variability of the *Puccinia triticina* population in Bulgaria during 2010-2012 and to compare the data to previous investigations.

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MATERIAL AND METHODS

The pathogen population from 10 regions in Bulgaria was studied. The samples were collected from different cultivars grown at varietal testing station, trial fields of research institutes and seed production fields. The samples were developed according to a standard methodology on the universally susceptible cultivar *Michigan amber* at stage second leaf (Dodov, 1934). The investigation was carried out under controlled climatic conditions optimal for the development of the pathogen: 20/15°C day/night, RH>75% and additional illumination for elongation of the photoperiod of 16/8h at 30000 lx.

During 2010-2012 a total of 221 isolates were analyzed. Urediniospores, of each isolate, were inoculated to differential set. The isogenic lines involved in the pathotype and genetic differentiation are given in Table 1.

Table 1. Isogenic lines used for pathotype and genetic differentiation

Lr genes	Pedigree	Origin	Identification number
Lr1	Tc*6/ Centenario	Wheat	RL 6003
Lr2a	Tc*6/ Webster	Wheat	RL 6016
Lr2b	Tc*6/ Carina	Wheat	RL 6019
Lr2c	Tc*6/ Loros	Wheat	RL 6047
Lr3	Tc*6/ Democrt	Wheat	RL 6002
Lr9	Transfer/ Tc*6	*Aegilops umbellulata*	RL 6010
Lr11	Tc*2/ Hussar	Wheat	RL 6053
Lr15	Tc*6/ Kenya W 1483	Wheat	RL 6052
Lr17	Klein Lucero/ Tc*6	Wheat	RL 6008
Lr19	Tc*7/ Translocation 4	*Agropyron elongatum*	RL 6040
Lr21	Tc*6/ RL5406xRL529	Wheat	RL 6043
Lr23	Lee 310/ Tc*6	*Triticum turgidum var. durum*	RL 6012
Lr24	Tc*6/ Agent	*Agropyron elongatum*	RL 6064
Lr26	Tc*6/ St-1-25	*Secale cereale*	RL 6078
Lr28	Tc*6/ C-77-1	*Aegilops speltoides*	RL 6079

The types of infection were determined 9-12 days after inoculation using a 0-4 scale suggested by Stakman et al. (1962); infection types 0, 0; 1, 2, 0-1, 0-2 expressed the resistant type of reaction, and infection 3-4 were considered an expression of susceptibility. The identification of pathotypes was based on a triplet code specifying the response of 15 monogenic lines in ascending order according to their gene designation (Limpert and Muller, 1994). The standard races were identified according to the International register (Johnston and Browder, 1966). Genetic analysis was done on the developed samples. The genetic formulae were represented as fractions with the efficient genes (resistant monogenic lines) as numerator and the inefficient genes (susceptible monogenic lines) as denominator, according to Green‘s scale (Green, 1965). The efficiency of the individual genes for resistance was calculated as percent of avirulent isolates from the total number of developed isolates.

RESULTS AND DISCUSSION

The results from the investigation on the race composition of *Puccinia triticina* in Bulgaria during 2010-2012 are given in Table 2.

Table 2. Current standard physiologic races of *Puccinia triticina* in Bulgaria during 2010-2012

N	Race	2010		2011		2012		Total	Average
		number	%	number	%	number	%		
1	167	10	13.9	4	6.7	8	8.9	22	10.0
2	77	31	43.1	22	36.7	37	41.6	90	40.7
3	57	30	41.6	34	56.6	39	43.8	103	46.6
4	176	1	1.4	-	-	-	-	1	0.4
5	149	-	-	-	-	5	5.6	5	2.3

Five standard physiological races were identified during 2010-2012. Another three races (157, 184 and 218) were present in the pathogen's population in the preceding period, although at low percent; these races were not detected during the current period (Ivanova, 2014). The dominant race under the conditions of Bulgaria was race 57 with 46.6%. This race was dominant during the previous 5-year period as well (Ivanova, 2012). The race has also been identified in Ukraine in 2002 (5.7%) and in 2003 (12.9%), but was not found in 2007 (Elyasi-Gomari and Mikhailovna, 2009). Second in distribution in Bulgaria was race 77 with 40.7%. In Bulgaria race 167 ranked third in distribution with 10%. During the preceding period the race was with higher percent of distribution and ranked second. Races 149 (2.3%) and 176 (0.4%) had lower percent of distribution during this period. In 2007, race 149 was third in distribution in the *Puccinia triticina* population in Ukraine. In a previous investigation (Ivanova, 2012) it was mentioned that the standard differential key has lost its ability to differentiate the great variability of the pathogen population and therefore we resorted to the use of the nomenclature suggested by COST 817, which included 15 isogenic lines.

Using this nomenclature, we identified 71 phenotypically different pathotypes during 2010-2012. The frequency of occurrence of these phenotypically distinct pathotypes is presented in Table 3. Twenty five of them were identified for the first time in the pathogen's population for the last 10 years. In 2010, the following pathotypes were identified for the first time in the Bulgarian population of *Puccinia triticina (Prt)*: 07772, 13763, 20772, 22776, 32763, 32773, 32776, 33571, 36771, 72571, 73571, 73732, 73776, 77773, with 1.4% distribution for each of them, as well as pathotypes 73575 and 77573 with 2.8% distribution. In 2011, pathotypes 63522, 63542, 72762, 73521 and 77562 were detected for the first time, with 1.7% for each of them. In 2012, pathotypes 32760 and 52763 occurred for the first time in the population with 1.1%, as well as pathotypes 32763 and 62763 with 2.2% distribution.

Table 3. Puccinia triticina pathotypes identified during 2010-2012

Prt- code	2010	2011	2012	Prt- code	2010	2011	2012
07772	1.4	-	-	63566	-	10.0	-
12762	-	-	3.3	63567	-	1.7	-
12773	-	1.7	-	63572	9.7	1.7	1.1
13763	1.4	-	-	63573	20.8	1.7	-
20772	1.4	-	-	63576	2.8	-	-
22573	1.4	-	-	63577	5.5	-	-
22773	1.4	-	-	63762	-	3.3	16.8
22776	1.4	-	-	63763	-	5,0	11.2
23573	1.4	-	-	63766	-	1.7	-
23773	1.4	-	1.1	63773	1.4	1.7	1.1
32760	-	-	1.1	63776	-	1.7	-
32762	-	-	3.3	63777	1.4	-	-
32763	1.4	-	2.2	72571	1.4	-	-
32773	1.4	-	-	72762	-	1.7	1.1
32776	1.4	-	-	72763	-	-	2.2
33571	1.4	-	-	73521	-	1.7	-
33762	-	-	1.1	73560	1.4	-	-
33772	-	1.7	-	73562	-	10.0	4.5
33773	1.4	-	-	73563	-	3.3	1.1
36771	1.4	-	-	73571	1.4	-	-
42762	-	-	1.1	73572	8.3	-	-
43562	-	-	1.1	73573	5.5	-	-
43572	1.4	-	-	73575	2.8	-	-
43577	2.8	-	-	73576	1.4	-	-
43763	-	-	1.1	73577	1.4	-	-
52763	-	-	1.1	73732	1.4	-	-
53763	-	-	1.1	73762	-	5.0	10.1
62562	-	-	2.2	73763	-	3.3	11.2
62572	1.4	-	1.1	73766	-	1.7	-
62762	-	-	1.1	73772	1.4	1.7	1.1
62763	-	-	1.1	73773	1.4	-	-
63522	-	1.7	-	73776	1.4	-	-
63542	-	1.7	-	77562	-	1.7	-
63552	-	1.7	-	77573	2.8	-	-
63562	-	28.3	7.8	77773	1.4	-	-
63563	-	3.3	6.7				

Pathotype 12762 was identified in 2012, with distribution 3.3%. This pathotype was registered for the first time in 2003 at very low percent of distribution (1.1%). During the rest of the years (2004-2011) it was not observed in the population of the pathogen. Pathotype 42762 was detected for the first time in 2001. Very low percent of occurrence was registered in 2002, and in the rest of the years till 2012 it was not present in the population. Pathotype 43763 was observed in 2001, 2002 and 2004, and then it reoccurred in 2012 with 1.1%. Pathotypes 53763 and 62762 were detected in the population in 2003, then in 2012 a low percent of distribution was registered for them (1.1%).

Pathotype 73576 was first identified in 2004, then again in 2010 (Ivanova, 2012).

Similar to pathotype 53763 it was pathotype 53762, which was present in the Czech population, as reported by Hanzalova (2010). In 2010, highest percent of distribution was registered for pathotype 63573 (20.8%), followed by pathotypes 63572 (9.7%) and 73572 (8.3%). Highest frequency of occurrence in 2011 was found in pathotype 63562 (28.3%), followed by pathotypes 63566 and 73562 (10%), and pathotypes 63763 and 73762 (5%). In 2012 the highest percentage of distribution is pathotype 63762 (16.8%), followed by pathotypes 63763 and 73763 (11.2%) and pathotype 73762 (10%). Only two of the pathotypes, 63773 and 73772, were present in the population during all three years of the investigation, their frequency of occurrence being within the low range from 1.1 to 1.7%. Table 4 shows data on the number of the three predominant races over locations during the investigated period.

Table 4. Number of isolates showing affiliation to the three predominant races during 2010-2012 over locations

Location	Race / Pathotype								
	63573			63562			63762		
	10	11	12	10	11	12	10	11	12
Burgas	2	0	0	0	0	1	0	0	2
Radnevo	2	0	0	0	2	0	0	0	1
Gorsky Izvor	2	0	0	0	1	0	0	0	2
Ognyanovo	3	0	0	0	1	0	0	1	2
Chepintsy	2	0	0	0	0	1	0	1	2
Selanovtsy	0	0	0	0	3	1	0	0	1
Pordim	0	0	0	0	4	0	0	0	0
Vardim	1	1	0	0	4	1	0	0	0
Brushlen	2	0	0	0	1	0	0	0	2
Dobrich	1	0	0	0	1	3	0	0	3

The predominant pathotype during 2010 was 63573, and, as shown on Table 4, it was found in almost all locations in Bulgaria, with the exception of Selanovtsy and Pordim, i.e. this pathotype was not detected in the north-west and the central part of Bulgaria in that year. Pathotype 63562 was predominant in 2011; it was also detected in almost all locations, with the exception of Burgas and Chepintsy, i.e. this pathotype was not found in the south-east and west part of the country. In 2012 single isolates of this pathotype were detected in these two locations. Pathotype 63762 was predominant in 2012. It was not registered at locations Pordim and Vardim. Isolates of this pathotype had not been found in the two preceding years and therefore it can be assumed that pathotype 63762 was not widespread in Central North Bulgaria during 2010-2012. The results from the investigation showed that there was high genetic variability of different virulence in the pathogen's population. During the investigated period, a total of 86 genetic formulae of virulence were determined. The most common genetic formulae during this period are given in Table 5.

Table 5. Percentage of the most frequent gene formulae of *P. triticina* in Bulgaria

Virulences (*Lr* genes)		Patho type	Year			Total for period	%
Efficient	Inefficient		2010	2011	2012		
9,19,28	1,2a,2b,2c,3,11,15,17,21,23,24,26	73763	-	2	10	12	18.2
9,19,24,28	1,2a,2b,2c,3,11,15,17,21,23,26	73762	-	3	9	12	18.2
9,15,24,28	1,2a,2b,2c,3,11,17,19,21,23,26	73572	6	-	-	6	10.0
1,9,15,28	2a,2b,2c,3,11,17,19,21,23,24,26	63573	13	-	-	13	19.7
1,9,19,28	2a,2b,2c,3,11,15,17,21,23,24,26	63763	-	3	11	14	21.2
1,9,15,24,28	2a,2b,2c,3,11,17,19,21,23,26	63572	8	1	1	10	15.2
1,9,15,19,24	2a,2b,2c,3,11,17,21,23,26,28	63566	1	7	-	8	12.1
9,15,19,24,28	1,2a,2b,2c,3,11,17,21,23,26	73562	-	6	4	10	15.2
1,9,19,24,28	2a,2b,2c,3,11,15,17,21,23,26	63762	-	2	14	16	24.2
1,9,15,19,24,28	2a,2b,2c,3,11,17,21,23,26	63562	-	19	6	25	37.9

Thirty-three new formulae were determined in 2010. The most frequent genetic formula in 2010 was 1, 9, 15, 28 / 2a, 2b, 2c, 3, 11, 17, 19, 21, 23, 26. This genetic formula was registered in 2007 as well, with 8.4% distribution (Ivanova, 2012). In 2011, 25 genetic formulae were determined, and the predominant one was 1, 9, 15, 19, 24, 28 / 2a, 2b, 2c, 3, 11, 17, 21, 23, 26. Pathotypes with this genetic formula were present in the population in 2002 with 2.2%, in 2004 with 0.9% and in 2005 with 0.8% (Ivanova, 2012). In 2012, 28 gene formulae were determined and the most widespread avirulent / virulent combination was 1, 9, 19, 24, 28 / 2a, 2b, 2c, 3, 11, 15, 17, 21, 23, 26. This gene combination was found in the population in 2001 (2.4%), in 2002 (15.4%), in 2003 (1.8%) and in 2008 (0.8%) (Ivanova, 2012).

In overall, during the 3-year period, the most numerous efficient gene combinations involved 6 genes. The genetic formulae with participation of six genes were 25 and the most frequent gene combination was 1, 9, 15, 19, 24, 28 / 2a, 2b, 2c, 3, 11, 17, 21, 23, 26 (37.9%), followed by the genetic combinations 1, 9, 19, 24, 28 / 2a, 2b, 2c, 3, 11, 15, 17, 21, 23, 26 (24.2%), which had 5 efficient genes, and 1, 9, 19, 28 / 2a, 2b, 2c, 3, 11, 15, 17, 21, 23, 24, 26 (21.2%), which was with 4 efficient genes.

The knowledge on the genetic structure reveals the evolutionary potential of the pathogen population. The genetic variability in the population leads to the occurrence of new virulent races capable of overcoming the resistance of the wheat cultivars. In this relation, annual investigations are carried out on the efficiency and the breeding value of the individual genes for resistance.

The data from the investigation showed that individual genes had invariably high or low efficiency, while the efficiency of others was highly variable (Table 6).

Until 2005, genes *Lr9* and *Lr19* were absolutely efficient. In 2005, pathotypes overcoming the resistance of these two genes were identified for the first time in the local population of *Puccinia triticina* in Bulgaria (Ivanova, 2012).

During the preceding period 2005-2009, single pathotypes overcoming the resistance of gene *Lr9* were also detected. The efficiency of gene *Lr19* during the preceding period was variable, dropping to 22% in 2007. During the current 3-year period, the efficiency of gene *Lr19* increased progressively from 62.5 to 95.5%.

Efficiency of *Lr19* in the recent years has been reported by Hanzalova et al. (2012). Hanzalova (2010) pointed out that virulence to *Lr19* in the Czech Republic was registered during 2005-2008.

Loss of efficiency of *Lr19* has been reported by Gultyaeva (2007) and Gultyaeva et al. (2000) for the Volga-Ural region, and in the Krasnodar region (Volkova, 2013) also reported overcoming of the resistance of this gene with about 2%. Virulence to *Lr19* has been found in Germany in 1999 and in Russia during 2001-2003 (Lind and Gultyaeva, 2007). Virulence to genes *Lr9*, *Lr19* and *Lr24* has not been detected in Latvia (Liatukas, 2003).

In Bulgaria, one hundred percent efficiency during 2010-2012 was demonstrated by genes *Lr22a, Lr22b* and *Lr43*. Highly efficient during the current period were genes *Lr3ka*, *Lr9*, *Lr19*, *Lr28*, *Lr41* and *Lr42*. Hanzalova et al. (2012) pointed out that only one isolate exhibited virulence to *Lr9* during 2009-2011, and virulence to *Lr28* was detected in all years of the investigation, but it was with low frequency. Genes *Lr1*, *Lr25*, *Lr40, Lr45*, *Lr47* and *Lr50* demonstrated very good efficiency in Bulgaria. Genes *Lr15, Lr20*, *Lr24* and *Lr48* showed good efficiency. The efficiency of genes *Lr32*, *Lr37*, *Lr44* and *Lr52* was satisfactory. Low efficiency was demonstrated by genes *Lr2a, Lr2b*, *Lr2c*, *Lr3*, *Lr10*, *Lr18*, *Lr21*, *Lr23*, *Lr26*, *Lr29*, *Lr33*, *Lr36*, *Lr38*, *Lr39* and *Lr60*, and genes *Lr11*, *Lr16*, *Lr17*, *Lr30* and *Lr35* were absolutely inefficient.

Table 6. Efficiency of the *Lr* genes during 2010-2012

Lr genes	2010		2011		2012		Total number	Average %
	Number of isolates	%	Number of isolates	%	Number of isolates	%		
Lr1	39	54.2	40	66.6	49	55.0	128	57.9
Lr2a	4	5.5	2	3.3	6	6.7	12	5.4
Lr2b	14	19.4	2	3.3	12	13.5	28	12.6
Lr2c	11	15.3	2	3.3	19	21.3	32	14.5
Lr3	3	4.2	0	0	0	0	3	1.4
Lr3ka	62	86.1	52	86.6	66	74.2	180	81.4
Lr9	67	93.0	59	98.3	89	100	215	97.3
Lr10	0	0	1	1.6	1	1.1	2	0.9
Lr11	0	0	0	0	0	0	0	0
Lr15	56	77.7	42	70.0	23	25.8	121	54.7
Lr16	0	0	0	0	0	0	0	0
Lr17	0	0	0	0	0	0	0	0
Lr18	0	0	1	1.6	0	0	1	0.4
Lr19	45	62.5	51	85.0	85	95.5	181	81.9
Lr20	-	-	53	88.3	59	66.3	112	50.7
Lr21	0	0	1	1.6	0	0	1	0.4
Lr22a	72	100	60	100	89	100	221	100
Lr22b	72	100	60	100	89	100	221	100
Lr23	1	1.4	2	3.3	0	0	3	1.4
Lr24	26	36.1	40	66.6	52	58.4	118	53.4
Lr25	-	-	60	100	76	85.4	136	61.5
Lr26	6	8.3	0	0	1	1.1	7	3.2
Lr27+31	37	51.4	45	75.0	21	23.6	103	46.6
Lr28	57	79.2	50	83.3	89	100	196	88.7
Lr29	-	-	0	0	1	1	1	0.4
Lr30	0	0	0	0	0	0	0	0

Lr genes	2010		2011		2012		Total number	Average %
	Number of isolates	%	Number of isolates	%	Number of isolates	%		
Lr32	69	95.8	0	0	0	0	69	31.2
Lr33	7	9.7	0	0	0	0	7	3.2
Lr35	0	0	0	0	0	0	0	0
Lr36	0	0	1	1.6	0	0	1	0.4
Lr37	24	33.3	21	35.0	15	16.8	60	27.1
Lr38	1	1.4	21	35.0	10	11.2	32	14.5
Lr39	7	9.7	4	6.6	2	2.2	13	5.8
Lr40	41	56.9	58	96.6	47	52.8	146	66.0
Lr41	60	83.3	42	70.0	80	89.8	182	82.4
Lr42	64	88.8	56	93.3	73	82.0	193	87.3
Lr43	72	100	60	100	89	100	221	100
Lr44	31	49.2	22	36.6	18	20.2	71	32.1
Lr45	50	69.4	41	68.3	42	47.2	133	60.2
Lr46	51	70.8	50	83.3	58	65.2	159	71.9
Lr47	42	87.5	-	-	88	98.8	130	58.8
Lr48	72	100	18	30.0	10	11.2	100	45.2
Lr50	71	98.6	4	6.6	-	-	75	33.9
Lr51	64	88.8	54	90.0	35	39.3	153	69.2
Lr52	36	50.0	12	20.0	20	22.5	68	30.7
Lr60	1	1.4	0	0	0	0	1	0.4

Virulence for many of these genes has been reported from all parts and zones worldwide where wheat is a main cereal crop (McIntosh et al., 1995; Kolmer et al., 2006; Liu and Chen, 2012). Hanzalova et al. (2012) reported virulent frequency of more than 80% in genes *Lr11*, *Lr15*, *Lr17* and *Lr21*. Virulence for gene *Lr21* was detected in 10 European countries. There was low virulence to this gene only in France (Mesterhazy et al., 2000). Kolmer (2011) reported that for the first time the resistance of this gene has been overcome in North America.

CONCLUSIONS

During 2010-2012, five standard physiological races of the cause agent of brown rust on wheat (*Puccinia triticina*) were identified in Bulgaria.

Seventy one phenotypically different pathotypes were found in the pathogen's population, the predominant ones being 63562, 63573 and 63762. The genetic variability was represented by 86 genetic formulae for virulence.

Twenty five new pathotypes were identified for the first time in the pathogen population of *Puccinia triticina* during 2010-2012.

The genes for resistance showed variable efficiency. Genes *Lr1, Lr3ka, Lr9, Lr19, Lr25, Lr28, Lr40, Lr41, Lr42, Lr45, Lr47* and *Lr50* demonstrated very good to high efficiency. Genes *Lr2a, Lr2b, Lr2c, Lr3, Lr10, Lr18, Lr21, Lr23, Lr26, Lr29, Lr33, Lr36, Lr38, Lr39* and *Lr60* exhibited low efficiency, while genes *Lr11, Lr16, Lr17, Lr30* and *Lr35* were absolutely inefficient during the investigated period. During this period immunity was provided by genes *Lr22a, Lr22b* and *Lr43.*

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