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Race and Virulence Dynamics of *Puccinia triticina* and Effectiveness of Lr genes in Bulgaria During 2005 – 2009

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Abstract

Durable and efficient resistance of wheat to *Puccinia triticina* can be ensured only through good knowledge on the structure of the pathogen population. During 2005-2009 the structure and variability in the population of brown rust was investigated at Dobroudja Agricultural Institute – Bulgaria. Eight standard races were identified, which was an indication that the great race variability of the pathogen can not be encompassed by using the standard differential set. Therefore the race variability was determined with the help of isogenic lines applying the nomenclature suggested at COST 817. During the investigated period, 172 phenotypically different pathotypes were identified, pathotypes 63562 (41%), 63573 (20%) and 63572 (19.4%) being predominant. The genetic variability within the population was represented through 236 gene formulae of virulence, 106 of which were detected for the first time in the population. Highly efficient were genes *Lr1, Lr9, Lr15, Lr28* and *Lr42*. During the period of investigation, pathotypes overcoming the resistance of gene *Lr19* were identified thus decreasing significantly its efficiency.

Key words: wheat; brown rust; pathotypes; virulence; genes

Ihtroduction

Brown rust on wheat caused by *Puccinia triticina* has highest economic significance among all rusts on wheat in Bulgaria. It is one of the most wide spread wheat disease worldwide. On the European continent, although without annual epidemic occurrence, epiphytoty is observed in separate years accompanied with significant decrease of yield. Such epidemics have been reported from Switzerland, Hungary, Germany, Romania, and from some regions in France and Italy (Winzeler et al., 2000).

The great variability in the virulence of the pathogen is the main source for overcoming the resistance of the cultivars and the propagation of new races. Therefore the investigation on the virulence variability of the pathogen population is a necessary prerequisite in breeding for resistance. Analysis on the virulence in the leaf rust population is being done in the USA and in most European countries. Masterhazy et al. (2000) have presented data on the variation in the *Puccinia triticina* population on the territory of 12 countries in Europe. In Slovakia and the Czech Republic such data have been provided by Bartoš (1996, 1998, 1999, 2001) and Hanzalova (2006, 2008, 2010). Pasquini et al. (2003) studied the distribution and frequency of occurrence of the pathotypes in the Italian population of the rust for a period of 12 years. Similar studies have been carried out also by Henriette Goyean et al. (2006) on the pathotypes of the population in France. In Bulgaria such studies have been done since 1930 and during the recent years the results have been published in a series of papers (Todorova, 1999; Todorova and Kiryakova, 2001; Karzhin, Stefcheva, Kiryakova, 2003; Stefcheva, Maneva, 2006; Kiryakova, 2007). At Dobroudja Agricultural Institute studies on the dynamics and the race and genetic composition of this pathogen are carried out annually.

The aim of this investigation was to present the variation of the pathogenic pathotypes in the structure of the *Puccinia triticina* population during 2005-2009 and the changes in the efficiency of the respective *Lr* genes.

Materials and Methods

The pathogen population from 7-10 regions of Bulgaria was studied; the samples were collected from cultivars grown at varietal testing stations, trial fields of research institutes and seed production fields and mass crops.

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.

During 2005-2009, a total of 455 isolates were analyzed. After propagation of the inoculum, the standard differential set and the individual isogenic lines were infected with each culture. The isogenic lines involved in the pathotype and genetic differentiation are given in Table 1. The pathotype differentiation included also 15 monogenic lines: *LrLr* 1, 2a, 2b, 2c, 3, 9, 11, 15, 17, 19, 21, 23, 24, 26 and 28; the genetic differentiation initially involved 21 monogenic lines and subsequently the new genes for resistance to the pathogen were added to the set. After inoculation, the plants and the differentiators were placed in moist chamber for 24h, and then were transferred to a greenhouse at temperature 20/15^{o} C day/night and additional illumination for elongation of the photoperiod: 16/8 h at 30000 lx.

Table 1. Isogenic lines used for pathotype and genetic differentiation

Lr genes	*Pedigree*	*Origin*	*Identification number*
Lr 1 ^{AB}	Tc*6/ Centenario	Wheat	RL 6003
Lr 2a ^{AB}	Tc*6/ Webster	Wheat	RL 6016
Lr 2b ^{AB}	Tc*6/ Carina	Wheat	RL 6019
Lr 2c ^{AB}	Tc*6/ Loros	Wheat	RL 6047
Lr 3 ^{AB}	Tc*6/ Democrt	Wheat	RL 6002
Lr 9 ^{AB}	Transfer/Tc*6	*Aegilops umbellulata*	RL 6010
Lr 10 ^{B}	Tc*6/ Exchange	Wheat	RL 6004
Lr 11 ^{AB}	Tc*2/ Hussar	Wheat	RL 6053
Lr 12 ^{B}	Exchange/ Tc*6	Wheat	RL 6011
Lr 13 ^{B}	Tc*6/ Frontana	Wheat	RL 6001
Lr 15 ^{AB}	Tc*6/ Kenya W 1483	Wheat	RL 6052
Lr 16 ^{B}	Tc*6/ Exchange	Wheat	RL 6005
Lr 17 ^{AB}	Klein Lucero/ Tc* 6	Wheat	RL 6008
Lr 18 ^{B}	Tc*7/Africa 43	*T. timopheevii*	RL 6009
Lr 19 ^{AB}	Tc*7/Translocation 4	*Agropyron elongatum*	RL 6040
Lr 21 ^{AB}	Tc*6/RL 5406xRL5289	Wheat	RL 6043
Lr 23 ^{AB}	Lee 310/ Tc*6	Triticum turgidum var. durum	RL 6012
Lr 24 ^{AB}	Tc*6/ Agent	*Agropyron elongatum*	RL 6064
Lr 26 ^{AB}	Tc*6/ St-1-25	*Secale cereale*	RL 6078
Lr 27+31 ^{B}	Gatcher	Wheat	Gatcher
Lr 28 ^{AB}	Tc*6/ C-77-1	*Aegilops speltoides*	RL 6079
Lr 30 ^{B}	Tc*6/Terenzio	Wheat	RL 6049
Lr 35 ^{B}	Tc*6/RL 5711	*Aegilops speltoides*	RL 5711
Lr 36 ^{B}	ER 84018	*Aegilops speltoides*	ER 84018
Lr 37 ^{B}	Tc*8/VPM	*Aegilops ventricosa*	RL 6081
Lr 38 ^{B}	Tc*6/T7 Kohn	*Thinopyrum intermedium*	RL 6097
Lr 39 ^{B}	KS86WGRCO2	*T. tauschii*	KS86WGRCO2
Lr 40 ^{B}	KS89WGRCO7	*T. tauschii*	KS89WGRCO7
Lr 41 ^{B}	KS90WGRCO10	*T. tauschii*	PI 549278
Lr 42 ^{B}	KS91WGRCO11	*T. tauschii*	PI 566668
Lr 43 ^{B}	KS92WGRCO16	*T. tauschii*	PI 592728
Lr 44 ^{B}	Tc*6/RL 7831	*T. spelta*	RL 6147
Lr 45 ^{B}		*Secale sereale*	RL 6144
Lr 46 ^{B}	Pavon F76	Wheat	PI 519847
Lr 47 ^{B}	Pavon	*Aegilops speltoides*	KS 90H45
Lr 48 ^{B}	CSP 44	Wheat	PI 520360
Lr 50 ^{B}	KS96WGRC36	*T.timopheevii subsp.armeniacum*	PI 604221
Lr 51 ^{B}	Neepava*6/A.speltoides	*Aegilops speltoides*	
Lr 52 ^{B}	Lr W	Wheat	RL 6107
Lr 60 ^{B}	Lr W_2	Wheat	

Lr ^{A} - Isogenic lines used for pathotype differentiation

Lr ^{B} - Isogenic lines used for genetic differentiation

The type of infection was read 9-12 days after inoculation according to a 4-degree scale suggested by Stakman et al., (1962), infection types 0, 0; 1, 2, 0-1, 0-2 expressing the resistant type of reaction, and infection type 3 - 4 considered an expression of susceptibility.

The monogenic lines were provided by Dr. A. Masterhazy (Cereal Research Institute - Szeged, Hungary) and Jim Kolmer (Cereal Disease Laboratory, USA). 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, 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) according to Green's scale as denominator.

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 analysis on the race composition of *Puccinia triticina* are given in Table 2.

Table 2. Current standard physiologic races of *Puccini triticina* in Bulgaria during 2005-2009

N	Race	2005		2006		2007		2008		2009		Total	%
		number	%	number	%	number.	%	number	%	Number	%		
1	167	73	57.9	40	44.4	38	31.9	5	8.3	6	10.0	162	35.6
2	77	23	18.3	13	14.4	12	10.1	37	61.6	20	33.3	105	23.1
3	57	17	13.5	34	37.7	65	54.6	17	28.3	33	55.0	166	36.5
4	176	11	8.7	2	2.2	0	0	0	0	0	0	13	2.8
5	149	1	0.8	1	1.1	4	3.4	0	0	0	0	6	1.3
6	157	1	0.8	0	0	0	0	0	0	0	0	1	0.2
7	218	0	0	0	0	0	0	1	1.6	0	0	1	0.2
8	184	0	0	0	0	0	0	0	0	1	1.7	1	0.2

During 2005-2009, race 57 was predominant among the standard races (36.5 %). This race had highest frequency of occurrence in 2007 and 2009 (Table 2). During the previous period of investigation (2002-2004) this race was fourth in occurrence (Kiryakova, 2007). Second in occurrence during 2005-2009 was race 167, with 35.6 % from the population of the pathogen. Race 77 ranked third with 23.1 %, while being second after race 167 during the previous 3 - year period of investigation. The other races had relatively small percent. In our previous publications it was pointed out that the race composition of the pathogen was rather narrowed; this, on the one hand, is due to the fact that the standard differentiators have lost their ability, an on the other – the fact that during the recent years the number of resistant cultivars used in practice increased (Kiryakova, 2007). In order to unify the results from the virulence analysis of the pathogen population for the EU countries, we decided to perform the present analysis on the basis of the isogenic lines although their application in this type of analysis has its drawbacks too.

The frequency of the isolates with virulence to the separate *Lr* genes is given in Table 3.

During 2005-2009 we identified 172 phenotypically different pathotypes of the cause agent of brown rust in the population of the pathogen. The results from the identification and the frequency of occurrence of these phenotypes by years are presented in Table 4. It was found that pathotype 63562 was prevalent (41 %), followed by pathotypes 63573 (20%) and 63572 (19.4%).The other pathotypes also occurred during individual years, but with low frequency. Nevertheless these pathotypes are a potential danger for wheat production and should be considered in breeding for resistance too.

Table 3. Frequency of isolates of *Puccinia triticina* in Bulgarian with virulence to isogenic lines of Thatcher wheat during 2005 – 2009

Lr lines	2005	2006	2007	2008	2009	Average %
Lr 1	17.5	20.0	16.8	60.0	35.0	29.8
Lr 2a	61.1	62.2	63.0	90.0	88.3	72.9
Lr 2b	77.7	57.7	53.8	71.6	88.3	69.8
Lr 2c	85.7	65.5	79.8	88.4	90.0	81.8
Lr 3	100	96.6	99.2	100	96.6	98.5
Lr 3ka	23.0	14.4	7.5	45.0	26.6	23.3
Lr 9	0.8	0	4.2	0	16.7	4.3
Lr 10	96.8	87.7	97.5	95.0	95.0	94.4
Lr 11	100	100	100	100	100	100
Lr 15	36.5	18.8	25.2	48.4	33.3	32.4
Lr 16	99.2	100	99.2	100	96.6	99.0
Lr 17	100	100	100	100	98.3	99.6
Lr 18	96.0	92.2	99.2	96.7	100	96.8
Lr 19	4.0	12.2	78.2	75.0	60.0	45.8
Lr 21	100	100	100	66.7	100	93.3
Lr 23	100	100	97.5	91.7	100	97.8
Lr 24	8.7	37.7	63.0	13.4	73.3	39.2
Lr 26	97.6	95.5	96.6	76.7	100	93.3
Lr 28	9.5	6.6	24.4	11.7	28.3	16.0
Lr 30	100	100	100	98.4	100	99.7
Lr 35				100	98.3	99.0
Lr 36	98.4	98.8	100	98.4	96.6	98.4
Lr 37			68.1	31.7	70.0	56.6
Lr 38			92.5	96.7	63.3	84.2
Lr 39			60.5	70.0	100	76.8
Lr 40			35.3	28.4	48.3	37.3
Lr 41			13.5	6.7	0	6.7
Lr 42	4.0	3.3	0	5	13.3	5.1
Lr 43			0	0	0	0
Lr 44					61.6	61.6
Lr 45					19.0	19.0
Lr 46			76.0	50.0	11.6	45.8
Lr 47					3.3	3.3
Lr 48			84.0	96.7	98.3	93.0
Lr 50			64.7	96.7	91.6	84.3
Lr 51					43.3	43.3
Lr 52					38.3	38.3
Lr 60					96.6	96.6
Number of testedisolates	126	90	119	60	60	

Hanzalova (2010) pointed out that most widespread in the Czech Republic were pathotypes 53762 and 43762. The predominant pathotypes on the territory of Hungary were 53502, 53522 and 53722, which constituted 31.7 % of the pathogen population, followed by pathotypes 43702 and 43722 with 23.2 %. Pathotype 53762 was detected in Germany and Russia (Lind and Gultyaeva, 2007). Some of the pathotypes were identified in the Bulgarian population of brown rust as well. Pathotype 43762 occupied 19.2 % from the population of the pathogen in Bulgaria during 2002-2004. During this period pathotypes 53762 and 43722 were also found in Bulgaria but their percent of occurrence was significantly lower (Kiryakova, 2007).

The genetic variability of the pathogen during the investigated period was represented with 236 gene formulae of virulence, through avirulent / virulent combination. Table 5 shows the most frequent genetic formulae of the *Puccinia triticina* pathotypes during 2005-2009.

During 2005, 33 gene formulae for virulence were identified, the new combinations according to the previous period being 16. Highest frequency in 2005 had the avirulent / virulent combination 1, 9, 15, 19, 24, 28, 42 / 2a, 2b, 2c, 3, 10, 11, 16, 17, 18, 21, 23, 26, 30, 36 with 17.5 % from the population. In 2006 this combination represented 5.5 % from the population, its frequency of occurrence significantly going down in the following years, and in 2009 pathotypes of this gene combination were not found. Nevertheless the above gene combination had the highest percent of frequency of occurrence for the investigated period (23.8 %). Second in frequency was the gene combination 1, 2a, 9, 15, 19, 24, 28 / 2b, 2c, 3, 10, 11, 16, 17, 18, 21, 23, 26, 30, 36 with 14.9 % in the population, and the combination 1, 2a, 2b, 2c, 9, 15, 19, 24, 28 / 3, 10, 11, 16, 17, 18, 21, 23, 26, 30, 36 ranked third with 12.4 %. This gene combination had highest percent of distribution in 2006 (10.0 %) among 62 gene formulae registered during this year. The gene formulae in 2007 were 73, gene combination 1, 9, 15, 28, 42 / 2a, 2b, 2c, 3, 11, 16, 17, 18, 19, 21, 23, 24, 26, 30, 36 being with the highest frequency (8.4%).

Table 4. Virulence phenotypes and frequency (%) of *Puccinia triticina* isolates in Bulgaria during 2005 - 2009

Prt-code	2005	2006	2007	2008	2009	Prt-code	2005	2006	2007	2008	2009	Prt-code	2005	2006	2007	2008	2009
00562	0	1.1	0	0	0	22562	0.8	3.3	0	0	0	32762	0	1.1	0	1.6	0
01562	0	1.1	0	0	0	22563	0.8	1.1	1.7	0	0	32770	0	0	0	1.6	0
02562	4.0	10.0	0.8	0	0	22566	0.8	0	0	0	0	32775	0	0	0.8	0	0
02563	0	2.2	0	0	0	22567	0	1.1	0	0	0	33522	0	0	0.8	0	0
02572	0	0	0.8	0	0	22572	0	0	1.7	0	0	33540	0	0	0	1.6	0
02573	0	0	1.7	0	0	22573	0	0	1.7	0	1.7	33560	0.8	0	0	0	0
02577	0	0	0.8	0	0	22576	0	0	0.8	0	0	33562	0	1.1	0	0	0
02742	0	1.1	0	0	0	22760	0	0	0	1.6	0	33563	0	0	0.8	0	0
02762	0.8	0	0	0	0	22762	1.6	1.1	0	0	0	33572	0	0	1.7	0	0
02763	0	1.1	0	1.6	0	22763	0	2.2	0	0	0	33573	0	0	0.8	0	0
02766	0.8	0	0	0	0	22773	0	0	0.8	0	0	33712	0	0	0	1.6	0
02767	0	1.1	0	0	0	22777	0	0	0.8	0	0	33762	0	1.1	0	0	0
02773	0	0	0.8	0	0	23552	0	0	0	3.3	0	33766	0	0	0.8	0	0
03562	4.0	2.2	0	0	0	23562	1.6	2.2	0.8	0	0	33772	0	0	0.8	0	0
03563	0	1.1	0	0	0	23563	0	1.1	1.7	0	0	33773	0	0	0.8	0	0
03566	0	0	0.8	0	0	23567	0	1.1	0	0	0	41562	0	1.1	0	0	0
03572	0	0	1.7	0	0	23572	0	0	0	1.6	0	42362	0.8	0	0	0	0
03573	0	0	3.4	0	0	23573	0	0	5.0	0	0	53773	0	0	0.8	0	0
03762	1.6	0	1.7	0	0	23576	0	0	0.8	0	0	42562	0,8	0	0	0	0
03763	0	1.1	0	0	0	23577	0	0	0.8	0	0	42771	0	0	0.8	0	0
03772	0	0	0.8	0	0	23756	0	0	0	1.6	0	43532	0	1.1	0	0	0
03773	0	0	0.8	0	0	23762	1.6	1.1	0	0	0	43561	0	1.1	0.8	0	0
07576	0	0	0.8	0	0	23772	0	0	0	3.3	0	43562	12.6	1.1	0	0	1.7
12562	0.8	0	0	0	0	23773	0	0	0.8	0	1.7	43563	0.8	3.3	1.7	0	1.7
12766	0	0	0	0	1.7	23776	0	0	0	1.6	0	43566	0.8	0	0	0	0
12772	0	0	0	1.6	0	23777	0	0	0.8	0	0	43567	0	0	0.8	0	0
12773	0	0	0.8	0	0	26573	0	0	0.8	0	0	43572	0.8	0	2.5	0	0
13572	0	1.1	0.8	0	0	27572	0	0	0.8	0	1.7	43573	0	1.1	5.8	0	1.7
13760	0.8	0	0	0	0	27736	0	0	0.8	0	0	43576	0	0	2.5	0	0
13772	0	0	0	3.3	0	32562	0.8	0	0	0	0	43577	0	0	0.8	0	0
13773	0	0	0.8	0	0	32567	0	0	0	0	1.7	43762	6.3	0	0	0	0
20562	0	1.1	0	0	0	32570	0	0	0	1.6	0	43766	1.6	1.1	0	0	0
22542	0	1.1	0	0	0	32573	0	0	0.8	0	0	43772	0	1.1	0	0	0

Table 4. (continued)

Prt-code	2005	2006	2007	2008	2009	Prt-code	2005	2006	2007	2008	2009	Prt-code	2005	2006	2007	2008	2009
43773	0	0	0.8	0	0	63566	1.6	0	0	0	0	73562	2.4	3.3	0	1.6	1.7
43777	0	0	0.8	0	0	63567	0	1.1	0.8	0	3.3	73563	1.6	0	0	1.6	1.7
45572	0	0	0	0	1.7	63570	0	0	0	1.6	0	73565	0	0	0	1.6	0
46763	0	1.1	0	0	0	63572	0	4.4	3.4	6.6	5.0	73566	0.8	0	0	0	0
52562	0	1.1	0	0	0	63573	0	0	8.4	1.6	1.,0	73567	0	1.1	0	0	0
53550	0	0	0	1.6	0	63576	0	0	1.7	0	0	73572	0	0	0.8	11.6	0
53560	0.8	0	0.8	0	0	63577	0	0	0.8	0	3.3	73573	0	0	0.8	0	8.3
53561	0	1.1	0	0	0	63732	0	0	0.8	0	0	73577	0	0	0	1.6	0
53562	0.8	0	0	0	0	63762	7.9	0	0.8	0	0	73702	0	0	0	1.6	0
53577	0	0	0.8	0	0	63763	3.2	0	0	0	0	73712	0	0	0	1.6	0
56677	0	0	0	0	1.7	63766	0.8	0	0.8	0	0	73720	0	0	0	1.6	0
60562	0	1.1	0	0	0	63767	0	0	0.8	0	0	73740	0	0	0	1.6	0
61563	0	1.1	0	0	0	63772	0	1.1	0	0	1.7	73742	0.8	0	0	0	0
61576	0	0	0.8	0	0	63773	0	0	2.5	0	6.6	73752	0	0	0	6.6	0
62562	0.8	0	0	0	1.7	63776	0	0	0.8	0	0	73760	0	0	0	1.6	0
62563	0	0	0.8	0	0	63777	0	0	1.7	0	0	73762	4.8	1.1	0	1.6	0
62572	0	1.1	0	0	0	66562	0	0	0.8	0	0	73763	1.6	5.5	0	0	3.3
62573	0	1.1	0	0	0	71777	0	0	0	0	1.7	73766	0.8	0	0	0	3.3
62574	0	0	0	1.6	0	72563	0	0	0	0	1.7	73767	0	0	0	0	3.3
63173	0	0	0	0	1.7	72577	0	0	0.8	0	0	73772	0	0	0	5.0	1.7
63510	0	0	0	1.6	0	73550	0	0	0	1.6	0	73773	0	0	0	0	3.3
63552	0	0	0	5.0	0	73552	0	0	0	1.6	0	73774	0	0	0	1.6	0
63562	23.0	12.2	0.8	3.3	1.7	73560	0	1.1	0	0	0	73777	0	0	0	1.6	0
63563	2.4	4.4	1.7	1.6	6.6	73561	0	1.1	0	0	0	77563	0	0	0	0	3.3
												77763	0	0	0	0	1.7

Table 5. Percentage of the most frequent gene formulae of *Puccinia triticina* in Bulgaria during 2005 – 2009

Virulence on Lr genes	Pathotype	Year					Average
		2005	2006	2007	2008	2009	
2a,2b,2c,3,10,11,16,17,18,21,23,26,30,36	63562	17.5	5.5	-	0.8	-	23.8
2b,2c,3,10,11,16,17,18,21,23,26,30,36	43562	12.7	2.2	-	-	-	14.9
3,10,11,16,17,18,21,23,26,30,36	02562	1.6	10.0	0.8	-	-	12.4
2a,2b,2c,3,11,16,17,18,19,21,23,24,26,30,36	63573	-	-	8.4	-	-	8.4
2a,3,10,11,16,17,18,21,23,26,30,36	22562	4.8	3.3	-	-	-	8.1
2b,2c,3,10,11,16,17,18,19,21,23,24,26,30,36	43573	-	1.1	5.8	-	-	6.9
2a,2b,2c,3,10,11,16,17,18,19,21,23,26,30,36	63572	-	2.2	3.4	-	-	6.7
2b,2c,3,10,11,16,17,18,21,23,24,26,30,36	43563	1.6	3.3	1.7	-	-	6.6
1,2a,2b,2c,3,10,11,15,16,17,1819,21,23,26,30,35,36,38,39	63572	-	-	-	6.6	-	6.6
2a,2b,2c,3,10,11,15,16,17,18,21,23,26,30,36	63762	5.5	-	0.8	-	-	6.3
2a,2c,3,10,11,16,17,18,19,21,23,24,26,30,36	23573	-	-	5.0	-	-	5.0
1,2a,2b,2c,3,10,11,15,16,17,18,19,21,23,26,30,35,36,38,39	73772	-	-	-	5.0	-	5.0
2a,2b,2c,3,10,11,16,17,18,19,21,23,26,30,35,36,37,38,39,40,44,48,50,60	63572	-	-	-	-	3.3	3.3

In 2008 the testing included isogenic lines *Lr37, Lr38, Lr39* and *Lr40,* and therefore 49 new gene formulae were registered. The gene combination 9, 24, 28, 37, 40, 41, 42 / 1, 2a, 2b, 2c, 3, 10, 11, 15, 16, 17, 18, 19, 21, 23, 26, 30, 35, 36, 38, 39 had highest frequency of occurrence (6.6 %). In 2009 isogenic lines *Lr43, Lr 44, Lr45, Lr46, Lr47, Lr48, Lr50, Lr51, Lr52* and *Lr60* were involved in the investigation, the new gene formulae registered during this year being 57. Only the gene formula 1, 9, 15, 24, 28, 41, 42, 43, 45, 46, 47, 51, 52 / 2a, 2b, 2c, 3, 10, 11, 16, 17, 18, 19, 21, 23, 26, 30, 35, 36, 37, 38, 39, 40, 44, 48, 50, 60 was with 3.3 % frequency of occurrence, while the percent of all other gene combinations was less than 2 % (Table 5).

In parallel with the investigation on the genetic differentiation of the pathogen, the variations in the efficiency of the used *Lr* genes were also studied. The data on the efficiency of the genes is presented in Figures 1 - 6. During the years of the investigation the efficiency of gene *Lr1* was comparatively high and stable, with the exception of 2008, when it was relatively good and was about 40 %. During the previous period of investigation (2002-2004), gene *Lr2a* was with moderate to high efficiency but in the first three years the efficiency was about the average or low during the last two years. The studies show that genes *Lr1* and *Lr2a* are highly efficient in the Czech Republic, Slovakia and Italy as well (Karzhin et al., 2003). Hanzalova (2010) reports recent increase in the virulence on gene *Lr1* in the Czech Republic and Hungary.

The efficiency of genes *Lr2b* and *Lr2c* was lower than the average in most of the years of investigation. In 2006 the two genes had moderate efficiency but during the rest of the years it was low. In the Czech Republic and Slovakia gene *Lr2b* was highly efficient, and gene *Lr2c* was expressed as having low efficiency in Italy and Hungary

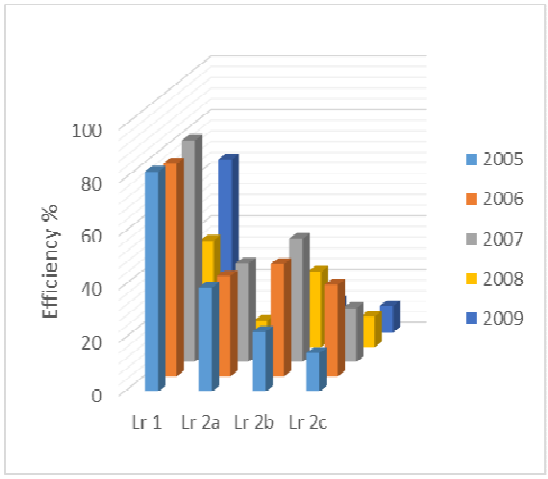

Fig. 1 Efficiency of genes *Lr1, Lr2a, Lr2b* and *Lr2c*

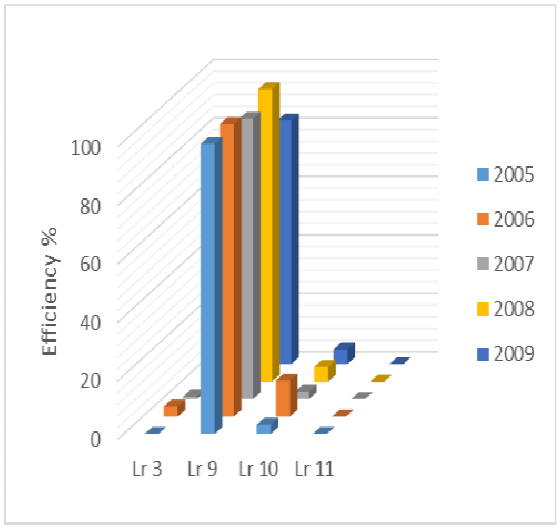

Fig. 2 Efficiency of genes *Lr3, Lr9, Lr10* and *Lr11*

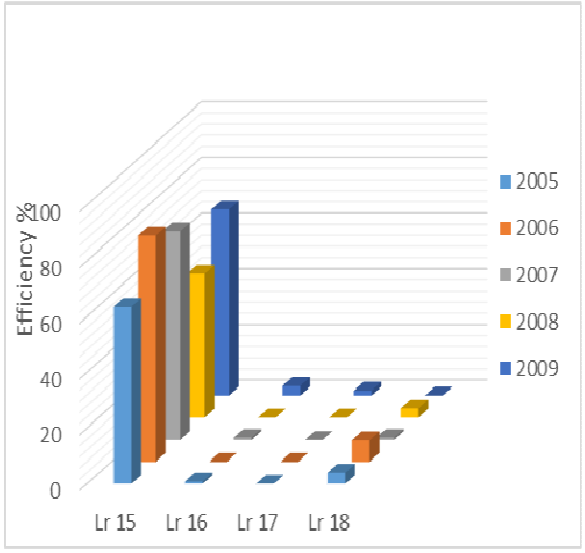

Fig. 3 Efficiency of genes *Lr15, Lr16, Lr17* and *Lr18*

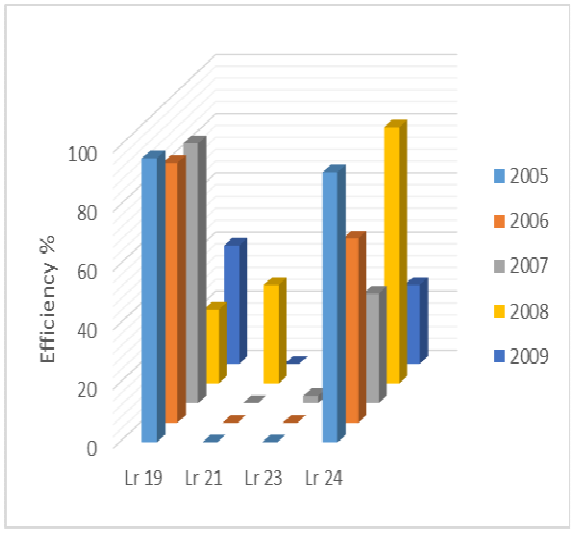

Fig. 4 Efficiency of genes *Lr19, Lr21, Lr23* and *Lr24*

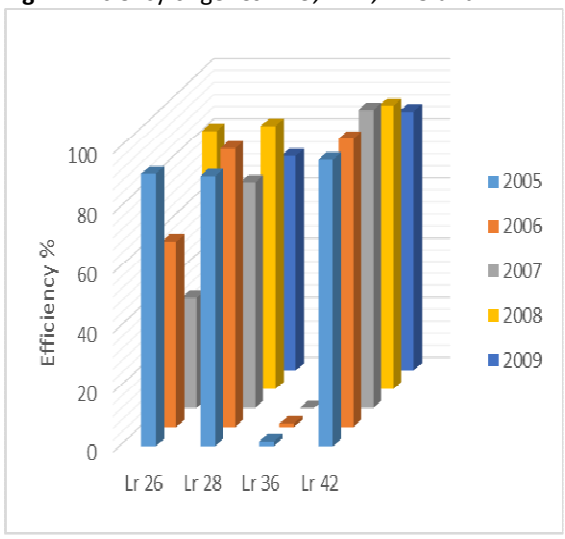

Fig. 5 Efficiency of genes *Lr26, Lr28, Lr 36 and Lr42*

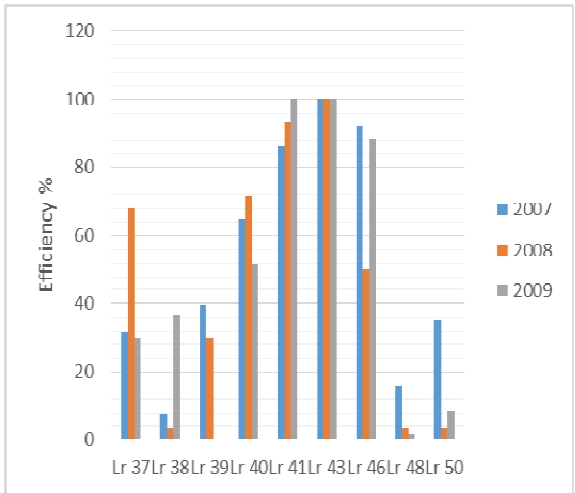

Fig. 6 Efficiency of genes *Lr37, Lr38,Lr39, Lr40, Lr41,*

Lr43, Lr46,Lr48 and *Lr50*Until 2005 genes *Lr9* and *Lr19* were entirely efficient. During this year pathotypes overcoming the resistance of these two genes were identified for the first time in the local population of *Puccinia triticina* in Bulgaria. There were similar reports from India, Mexico, USA and Russia (Sibikeev et al., 1996; Bhardwaj et al., 2005; Huerta – Espino, R. Singh, 1994, Kolmer et al., 2007). In India the new pathotypes have overcome *Lr19* with about 2 %. Markelova (2007) published data showing that in 1996 the frequency of the pathotypes overcoming the resistance of gene *Lr19* in the region along Volga river was 4.6 %, in 1998 increased to 33.3 %, and in 2001 severe brown rust epiphytoty was observed and the frequency of pathotypes overcoming the resistance of this gene reached 100 % thus proving that this gene had practically lost its efficiency. The reason for this situation is considered the distribution in production of the spring wheat cultivars L 503 and Dobrynya carrying gene *Lr19* which caused the occurrence of new pathotypes in the population with complementary genes for virulence; subsequently this gene lost its efficiency. Lind and Gultyaeva (2007) also reported overcoming of the resistance of this gene in the regions of Ural and Volga-Vyatka. Hanzalova (2010) presented data on the variations in the brown rust population in the Czech Republic during 2005 – 2008 and pointed out that isolates overcoming the resistance of gene *Lr9* have not been found, but one isolate was detected in 2005 and another two in 2008 which have overcome the resistance of gene *Lr19*. In her opinion the reason for this virulence is a new mutation.

In the Bulgarian population of the pathogen pathotypes overcoming the resistance of gene *Lr9* were not identified in 2006, but in 2007 the efficiency of this gene decreased to 95.8 %, which was an indication that single pathotypes were overcoming the resistance of *Lr9*. The percent of virulent isolates on this gene was higher in 2009: 16.7 %. The efficiency of gene *Lr19* continuously decreased: in 2006 it was 88.9 %, in 2007 it was as low as 21.8%, and in the next two years it gradually increased to 40 % in 2009. Kolmer et al. (2007) reported virulence on gene *Lr9* lower than 5 % during 1985 – 1999, which sharply increased to 30 % in 2004 in the south west part of USA where common winter wheat cultivars carrying this gene are grown more extensively.

Other genes expressed as highly efficient under the conditions of our country during 2005 – 2009 were genes *Lr24*, *Lr28* and *Lr42*. Since 2006 the efficiency of gene *Lr24* started decreasing and in 2007 and 2009 its efficiency was about moderate.In the west European countries this gene was identified as absolutely efficient and demonstrated sufficient efficiency in Belarus. In the Czech Republic the virulence on this gene was rare (Hanzalova, 2010). Gene *Lr15* demonstrated very good efficiency under our conditions (60-80 %). Gene *Lr10* was identified as low-efficient in Bulgaria and Italy, while it was expressed as highly efficient in Hungary, and with very good efficiency in Belarus. Genes *Lr Lr 3, 16, 18, 21, 23, 26* and *36* demonstrated low efficiency during the years of the investigation, and genes *Lr Lr 11, 17* and *30* were totally inefficient. In the last two years of the investigation new genes for resistance to the pathogen were included and genes *Lr41* and *Lr 43* demonstrated absolute efficiency during both years of the investigation. Genes *Lr37, Lr40* and *Lr46* demonstrated good efficiency during the first year, and the efficiency of genes *Lr37* and *Lr40* decreased during the second years, while the efficiency of gene *Lr46* sharply increased to more than 80 %. Genes *Lr38, Lr39, Lr48* and *Lr50* proved low-efficient.

Conclusions

During 2005 – 2009 eight standard physiological races of the cause agent of brown rust on wheat *Puccinia triticina* were identified. The small number of identified races shows that the standard differentiation sets have lost their ability and this imposes the necessity of a new differentiation set.

The analysis on the structure of the population of *Puccinia triticina* was carried out according to the modifications and methodologies accepted at COST 817. In the population of the pathogen, 172 phenotypically different pathotypes were determined, pathotypes 63562, 63573 and 63572 being predominant. The genetic variability was represented with 236 genetic formulae of virulence, 106 of which were identified for the first time in the population.

The genes for resistance demonstrated variable efficiency. Genes *Lr Lr 1, 9, 15, 28* and *42* showed very good to high efficiency. The efficiency of gene *Lr19* started decreasingly sharply, which was an indication for occurrence of changes in the genetic spectrum of the pathogen. Genes *Lr Lr 3, 16, 18, 21, 23, 26* and *36* demonstrated low efficiency, and genes *Lr Lr 11, 17* and *30* were totally inefficient.

Among the new genes, *Lr41* and *Lr43* exhibited absolute efficiency, and gene *Lr46* was highly efficient. Moderately efficient were genes *Lr37*

and *Lr40,* and genes *Lr38, Lr39, Lr48* and *Lr50* were determined as having low efficiency.

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