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Physiologic specialization of *Puccinia triticina* Erikss. and effectiveness of *Lr* genes in Bulgaria

Vanya Ivanova*, Stilyana Velikova and Dimitrina Nikolova

Dobrudzha Agricultural Institute, 9520 General Toshevo, Bulgaria
**Corresponding author:* vkiryakova@yahoo.com

Abstract

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Leaf rust caused by *Puccinia triticina* Erikss. is the most common diseases on wheat in Bulgaria. Monitoring on the pathogen population is carried out annually, and the information concerning the changes occurring in it and the data on the efficiency of the genes for resistance are helpful for the breeders. Different systems for pathotype identification are used in different parts of the world. This investigation considers the pathogen variability of *Puccinia triticina* in Bulgaria during 2015-2016. Twenty isogenic lines were involved, which were developed on the basis of cultivar *Tatcher*; the identification of the pathotypes is presented according to the North American nomenclature. One hundred twenty-nine isolates were analyzed, which were collected from seven different agro ecological zones of Bulgaria, and 42 phenotypically different pathotypes were identified. Three pathotypes were predominant in 2015: PKTTS (29%), PHTTS (13%) and MKTTS (11.6%). The most widespread pathotype in 2016 was TKTTN (16.6%). The distribution of the pathotypes over agro ecological zones was uneven. The efficiency of the genes for resistance was investigated and it was determined that genes *Lr 9* and *Lr 19* maintained their full efficiency, and genes *Lr 22A, Lr 22B, Lr 28, Lr 41, Lr 43* and *Lr 47* were with high efficiency.

Keywords: leaf rust; pathotypes; *Puccinia triticina; Lr* genes; effectiveness

Introduction

Leaf rust caused by *Puccinia triticina* is one of the most widespread diseases on wheat in Bulgaria. It occurs annually, and in years when the conditions are favorable for the propagation of the pathogen it can cause significant damage. The intensification of plant breeding in the recent years is related to the development of genotypes which are not only highly productive but also resistant to biotic and abiotic stress factors of the environment.

The successful development of new wheat varieties resistant to this disease depends on a number of factors. The population of the pathogen is characterized with high virulence potential represented by pathotypes with annual large-scale or more limited occurrence, which are nevertheless a potential risk for the wheat cultivars grown in Bulgaria. Therefore, the monitoring on the intra-population structure of *Puccinia triticina* is important. It allows reading the frequency of occurrence of the individual pathotypes, their virulence and dynamics of distribution. The environmental factors significantly influence the ratio of the genotypes in the population. Populations with different morphocultural and physiological and biochemical traits are formed in the different ecological zones. The environment may affect the propagation rate of the pathogen, the rate of the mutation process and the intensity of the genetic exchange. Therefore, the favorable conditions ensure higher numbers in the population and a greater genetic variability. On the other hand, the study on the efficiency of the individual genes for resistance to leaf rust provides information to the breeders about which genes

are highly efficient during the respective period and whether they can be involved in the breeding programs for limiting the infection at the early stages of the vegetative growth of the plants. The virulence potential of the pathogen and of the changes, which occur in the populations, are regularly followed in many places worldwide: the USA, Canada (Kolmer,1995; 2017) and South America (Kolmer et al., 2011), Western and Eastern Europe (Masterhazy et al., 2000; Mantovani, 2010; Goyeau, 2011; Kolmer et al., 2013; Hanzalova & Bartos, 2014), the Middle East, Central and Southern Asia (Kolmer et al., 2011; Prasad et al., 2017), Africa (Pretorius, 2015; Walid et al., 2016; Walid, 2018) and Australia (Park, 2016).

The racial and genetic composition has been investigated in Bulgaria since 1930 (Daskalov, 1930). Every year, a large number of pathotypes of different virulence are identified in Bulgaria, and all these investigations are presented in a series of papers: Todorova (1999); Todorova & Kiryakova (2000, 2001); Karzhin et al. (2003); Stefcheva & Maneva (2006); Kiryakova (2007); Ivanova (2012, 2014).

The aim of this investigation was to analyze the virulence of the pathogen population of *Puccinia triticina* in Bulgaria during 2015-2016 and monitor the efficiency of the *Lr* genes, which determine the resistance to this pathogen.

Material and Methods

The investigation was carried out at the Plant Pathology Laboratory of Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, during 2015-2016. One hundred and twenty-nine monoracial isolates were developed out of 43 samples. The analysis of the pathogen's virulence was performed according to the methodology adopted at the Plant Pathology Laboratory.

Collection of wheat leaf rust samples

Samples from leaves infected with *Puccinia triticina* were collected from different wheat cultivars and lines, as well as from mass crops in seven agro ecological regions in Bulgaria. The samples were placed in paper bags with designated date and location of collecting. The samples were then dried at room temperature and stored in an exiccator at 4°C until the performance of the race composition analysis.

Multiplication of mono pustules

The samples were processed during the winter months, from November to March of the following year. The samples collected in the summer were placed in Petri dishes on filter paper, then they were sprayed with water and left for 18-20 hours at room temperature until swelling of the sores, followed by inoculation of the universally susceptible cultivar *Michigan amber*. Seven to nine days following inoculation, when the sores were well expressed, three single well-developed apical sores were selected, and each of them was transferred to 7-day seedlings of the susceptible cultivar *Michigan amber*, so that 3 single isolates were formed from each sample. After inoculation, the plants were sprayed with water using a vaporizer, and were covered with a glass insulator. The plants thus inoculated were left for 24 hours in a dark chamber at 18-22°C. After that the inoculated plants were transferred to a growth chamber under controlled conditions (20/15°C day/night, RH>75% and additional illumination for elongation of the photoperiod 16/8h at 3000 lx.) favorable for the development of the plants and the pathogen. For better sporulation, Maleic hydrazide 97% was used (1g per 3 l of water, application: 100 ml/pot). The inoculation of the susceptible cultivar *Michigan amber* with each isolate was repeated several times, until sufficient inoculum was accumulated for infection of the differential set.

Designation of races

The infection type (IT) was read 7-9 days after the inoculation of the differential set with each of the isolates using a 0-4 scale according to Stakman et al. (1962). Infection types 0, 0;, 1, 2, 0-1, 0-2 represented the resistant type of reaction, and infection 3-4 were considered an expression of susceptibility. The differential set included 20 isogenic lines, given in Table 1, which were grouped in 5 sets: 1st set - *Lr1, Lr2a, Lr2c, Lr3;* 2nd set – *Lr9, Lr16, Lr24, Lr26;* 3rd set – *Lr3ka, Lr11, Lr17, Lr30;* 4th set – *LrB, Lr10, Lr14a, Lr18;* 5th set – *Lr3bg, Lr14b, Lr20, Lr28.*

Each isolate was given a five-letter code based on virulence/avirulence to each of the five sets of four differentials, adapted from the North American Nomenclature for virulence in *P. triticina* (Long & Kolmer, 1989; Kolmer et al., 2013). The nomenclature of the *P. triticina* races on 20 differential lines is presented in Table 2.

Results and Discussion

During the investigated period, a total of 129 isolates were analyzed (69 in 2015 and 60 in 2016). Using the North American nomenclature, 23 phenotypically different pathotypes were identified in 2015, and in 2016 they were 25. Six pathotypes were identified during both years of study. The distribution of the pathotypes by frequency of occurrence during the period of investigation is given in Table 3.

Pathotype PKTTS was with the highest percent of distribution in 2015 (29.0%). It was registered in all seven agro ecological regions of Bulgaria (Table 4). The second most

Table 1. Isogenic lines used for pathotype differentiation

Lr genes	Pedigree	Origin	Identification number
Lr 1	Tc*6/ Centenario	Wheat	RL 6003
Lr 2a	Tc*6/ Webster	Wheat	RL 6016
Lr 2c	Tc*6/ Loros	Wheat	RL 6047
Lr 3	Tc*6/ Democrat	Wheat	RL 6002
Lr 3ka	Klein Aniversario/6*T	Wheat	RL 6007
Lr 3bg	Bage/8*Tc	Wheat	RL 6042
Lr 9	Transfer/Tc*6	*Aegilops umbellulata*	RL 6010
Lr 10	Tc*6/ Exchange	Wheat	RL 6004
Lr 11	Tc*2/ Hussar	Wheat	RL 6053
Lr 14a	Selkirk/6*Tc	Wheat	RL 6013
Lr 14b	Maria Eskobar/6*Tc	Wheat	RL 6006
Lr 16	Exchange/ Tc* 6	Wheat	RL 6005
Lr 17	Klein Lucero/ Tc* 6	Wheat	RL 6008
Lr 18	South Africa43/7*Tc	*T.timofeevii*	RL 6009
Lr 20	Tc*6/RL5406xRL529	Wheat	RL 6092
Lr 24	Tc*6/ Agent	*Agropyron elongatum*	RL 6064
Lr 26	Tc*6/ St-1-25	*Secale cereale*	RL 6078
Lr 28	Tc*6/ C-77-1	*Aegilops speltoides*	RL 6079
Lr 30	Tc*6/ Terencio	Wheat	RL 6049
Lr B	Tc*6/Carina	Wheat	RL 6051

Table 2. Nomenclature of *P. triticina* races on 20 differential hosts in ordered sets of five Pt code

PR code	Host set	Infection type (ITs) produced on differential Lr lines			
	Host set 1 Host set 2 Host set 3 Host set 4 Host set 5	1 9 3ka B 3bg	2a 16 11 10 14b	2c 24 17 14a 20	3 26 30 18 28
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

Source: Long & Kolmer, 1989; Kolmer et al., 2013

common pathotype on the territory of Bulgaria in 2015 was PHTTS (13.0%). It was identified in six of the above regions and was not found in samples from South-Eastern Bulgaria. The third pathotype occurring in a greater percent of the samples in 2015 was MKTTS with 11.6%. It was determined in three agro ecological regions - Eastern, Central-Southern and North-Western Bulgaria; its percent of distribution was highest in Eastern Bulgaria (8.7%).

The higher or lower percent of development depended on the specific agro-ecology in each of the regions. In 2016, the highest percent of distribution was demonstrated by pathotype TKTTN (16.6%). It was distributed in 5 of the 7 regions in Bulgaria, in the north, south-east, west and north east parts of the country (Table 4).

Pathotypes PKTTN and PKTTS were with 15% of distribution in 2016. The first pathotype was identified in samples from South-Eastern, Western, Central Northern and North-Eastern Bulgaria, and the second was found in Central Southern, Western, North-Western, Central Northern and North-Eastern Bulgaria. In Eastern Bulgaria, 11 virulent pathotypes were identified in the 23 developed isolates; their percentage is given in Table 4. In 2016, 9 isolates were identified from the same agro ecological region, and 8 virulent pathotypes were determined. The predominant pathotype in 2015 in this agro ecological region was PKTTS (10.2%), followed by pathotypes MKTTS (8.7%) and TKTTS (4.3%). In 2016, pathotype TKTTN (3.3%) was with the highest per-

cent of distribution. All other phenotypes identified in that year in that respective zone were with 1.6% distribution. In 2015, two virulent pathotypes, THTTS and PKTTS, were determined from isolates collected in South-Eastern Bulgaria, both with 1.4% distribution. In 2016, 7 virulent phenotypes were identified out of 9 tested isolates; pathotype TKTTN was with the highest percent of distribution (5.0%).

In Central North Bulgaria, 10 virulent phenotypes were identified in 18 developed isolates in 2015, pathotype PKTTS having the highest percent of distribution (10.2%), and in 2016, 8 virulent pathotypes were found in 12 tested isolates, the predominant ones being PHTTS and PKTTS with 5.0% distribution. In Western Bulgaria during the two years of investigation, 6 isolates were tested in each year and 6 virulent phenotypes were respectively identified.

The data from the investigation revealed that in this agro ecological zone all pathotypes were evenly distributed in both years (Table 4). Six phenotypes were identified from the samples collected in North-Western Bulgaria in 2015, all evenly distributed, and in 2016 four phenotypes were

Table 3. Frequency of *Puccinia triticina* pathotypes in Bulgaria during 2015-2016

2015				2016			
Pathotype	%	Pathotype	%	Pathotype	%	Pathotype	%
PKTTS	29.0	PFTTS	1.4	TKTTN	16.6	TFTRQ	1.6
PHTTS	13.0	MKTTG	1.4	PKTTN	15.0	TKKTJ	1.6
MKTTS	11.6	MHTTQ	1.4	PKTTS	15.0	TKTTT	1.6
TKTTS	7.2	MFTTS	1.4	PHTTS	10.0	THTTS	1.6
MHTTS	5.8	MHTKS	1.4	TKTTS	6.6	THTTD	1.6
MHTTT	2.9	LKTTS	1.4	PKKTS	3.3	MKTTL	1.6
KKTTS	2.9	THRTS	1.4	TKTTL	3.3	PHTTN	1.6
FHTTS	2.9	THTTS	1.4	PKTTP	3.3	PHTTJ	1.6
TKTTT	2.9	NFTTS	1.4	PKTTQ	1.6	TKRTP	1.6
FKTTS	2.9	NCTTJ	1.4	PKTTT	1.6	PKTKN	1.6
PKTTQ	1.4			PFKTS	1.6	MKTTN	1.6
PHTPS	1.4			FHSRT	1.6	TKKTN	1.6
PHTMS	1.4			MKTTQ	1.6		

Table 4. Agro ecological zone, phenotype and percent of distribution (%) during 2015-2016

	East Bulgaria		South-Eastern Bulgaria		Central-Southern Bulgaria		West Bulgaria		North-Western Bulgaria		Central-Northern Bulgaria		North-Eastern Bulgaria	
	phenotype	%	phenotype	%	phenotype	%	phenotype	%	phenotype	%	phenotype	%	phenotype	%
2015	TKTTT	1.4	THTTS	1.4	MHTTS	1.4	TKTTT	1.4	MHTTS	1.4	PHTTS	4.3	PHTTS	1.4
	MHTTS	1.4	PKTTS	1.4	PKTTS	10.2	THRTS	1.4	MHTTQ	1.4	PKTTS	2.9	FHTTS	2.9
	PKTTS	10.2			LKTTS	1.4	TKTTS	1.4	MKTTS	1.4	MHTTS	1.4	PKTTS	1.4
	MKTTS	8.7			MKTTG	1.4	MHTKS	1.4	PKTTS	1.4	NCTTJ	1.4	FKTTS	1.4
	TKTTS	4.3			PHTTS	2.9	PKTTS	1.4	MHTTT	1.4	NFTTS	1.4	KKTTS	1.4
	KKTTS	1.4			PKTTQ	1.4	PHTTS	1.4	PHTTS	1.4				
	FKTTS	1.4			MFTTS	1.4								
	PHTPS	1.4			PFTTS	1.4								
	PHTMS	1.4			TKTTS	2.9								
	PHTTS	1.4			MKTTS	1.4								
2016	TKTTT	1.6	TKTTN	5.0	TKRTJ	1.6	PHTTS	1.6	PKTTS	3.3	PKTTN	5.0	PKTTN	3.3
	TKTTL	1.6	TKTTL	1.6	PKTTS	5.0	FHSRT	1.6	PKKTS	1.6	TKTTS	3.3	TKTTN	5.0
	TKTTN	3.3	PKTTP	1.6	PHTTS	5.0	PKTTT	1.6	TKTTN	3.3	PKTTS	5.0	PKTTP	1.6
	TKKTN	1.6	PKTTN	1.6	MKTTQ	1.6	TKTTN	1.6	MKTTN	1.6	PHTTS	3.3	PKTTS	1.6
	PHTTN	1.6	MKTTL	1.6	TFTRQ	1.6	PKTTN	1.6					PFKTS	1.6
	PHTTJ	1.6	TKRTP	1.6	PKTTQ	1.6	PKTTS	1.6					PKKTS	1.6
	THTTS	1.6	PKTKN	1.6	THTTD	1.6								
	TKTTS	1.6			TKTTS	1.6								

determined (Table 4). Five virulent phenotypes were identified in Central East Bulgaria, pathotype PHTTS being with the highest distribution (4.3%); in 2016, 4 phenotypes were determined, pathotypes PKTTN and PKTTS having higher percent of distribution (5.0%). In North-Eastern Bulgaria, 5 pathotypes were identified in 2015, the predominant pathotype in this zone being FHTTS (2.9%), while in 2016, six virulent phenotypes were found, the predominant one being pathotype TKTTN (5.0%). The percent ratio of the pathotypes is given in Table 4.

Pathotype PKTTS, which was predominant in 2015, was with well-expressed virulence with regard to genes *Lr1, Lr2c, Lr3, Lr3ka, Lr3bg, Lr10, Lr11, Lr14a, Lr14b, Lr16, Lr17, Lr18, Lr20, Lr24, Lr26, Lr30, LrB,* and pathotype TKTTN, which was predominant in 2016, was with well expressed virulence for genes *Lr1, Lr2a, Lr2c, Lr3, Lr3ka, Lr3bg, Lr10, Lr11, Lr14a, Lr16, Lr17, Lr18, Lr20, Lr24, Lr26, Lr30, LrB.* Two of the pathotypes predominant in India (THTTS and MHTTS), reported by Bhardwaj et al. (2016), were identified in the Bulgarian population of leaf rust during 2015-2016, too.

The efficiency of the genes for resistance to leaf rust in 2015 and 2016 is presented in Table 5.

The data from the investigation revealed that individual genes had constant high or low efficiency, while the efficiency of other genes was highly variable (Table 5). Genes *Lr9* and *Lr19* maintained their absolute efficiency during the current period while during previous periods there were certain pathotypes, which were overcoming the resistance of these two strong genes (Ivanova, 2014).

Extreme reduction in the efficiency of gene *Lr19* on the territory of Bulgaria was reported in 2007 reaching up to 22% (Ivanova, 2012, 2014). The overcoming of the resistance of these two genes was probably due to the transfer of more aggressive races with the air currents coming from the south. Hanzalova (2012) pointed out that only one isolate showed virulence to *Lr9* during 2009-2011, while virulence to *Lr28* has been identified during all the years of the investigation, although with low frequency.

Huerta Espino (1994) reported overcoming of the resistance of gene *Lr19* on the territory of Mexico. Such virulence was first discovered on the territory of Russia in 1996, as reported by Sibikeev et al. Virulence to *Lr19* was found in India in 2004, but during 2008-2013 such virulence was not determined for genes *Lr9, Lr24, Lr25, Lr32, Lr39* and *Lr45* (Bhardwaj et al., 2016).

Table 5. Efficiency of *Lr* genes to *Puccinia triticina* in Bulgaria during 2015-2016

Lr-genes	2015		2016		Total number	Average, %	*Lr*-genes	2015		2016		Total number	Average, %
	Avirulence isolates	%	Avirulence isolates	%				Avirulence isolates	%	Avirulence isolates	%		
1	6	8.6	1	1.6	7	5.4	27+31	7	10.1	3	5.0	10	7.8
2a	58	84.0	39	65.0	97	75.2	28	64	92.7	52	86.6	116	89.9
2b	40	57.9	15	25.0	55	42.6	29	27	39.1	11	18.3	38	29.5
2c	19	27.5	3	5.0	22	17.1	30	0	0	1	1.6	1	0.8
3	3	4.3	0	0	3	2.3	36	11	15.9	0	0	11	8.5
3ка	0	0	4	6.6	4	3.1	37	2	2.9	0	0	2	1.6
9	69	100	60	100	129	100	38	7	10.1	3	5.0	10	7.8
10	2	2.8	0	0	2	1.5	39	14	20.3	7	11.6	21	16.3
11	0	0	0	0	0	0	40	38	55.0	20	33.3	58	45.0
15	0	0	5	8.3	5	3.9	41	67	97.1	59	98.3	126	97.7
16	4	5.7	1	1.6	5	3.9	42	52	75.4	40	66.6	92	71.3
17	1	1.4	2	3.3	3	2.3	43	67	97.1	59	98.3	126	97.7
18	0	0	7	11.6	7	5.4	44	12	17.4	10	16.6	22	17.1
19	69	100	60	100	129	100	45	10	14.5	5	8.3	15	11.6
20	4	5.7	6	10.0	10	7.8	46	15	21.7	27	45.0	42	32.6
21	3	4.3	1	1.6	4	3.1	47	50	72.5	57	95.0	107	83.0
22 A	67	97.1	59	98.3	126	97.7	48	22	31.8	12	20.0	34	26.4
22 B	67	97.1	59	98.3	126	97.7	51	23	33.3	3	5.0	26	20.2
23	15	21.7	3	5.0	18	14.0	52	13	18.8	4	6.6	17	13.2
24	25	36.2	12	20.0	37	28.7	60	8	11.6	1	1.6	9	7.0
25	58	84.0	40	66.6	98	76.0	63	28	40.6	3	5.0	31	24.0
26	0	0	0	0	0	0	64	10	14.5	0	0	10	7.8

In some European countries such as Switzerland, the Netherland, South France and Germany, virulence to gene *Lr9* has not been found (Walid, 2018). This gene is also efficient against the predominant leaf rust races in Northern Ireland, as reported by Dhillon and Dhaliwal (2011). Huerta Espino et al. (2008) reported that in Mexico 7 new virulent pathotypes were discovered, which overcame the resistance of gene *Lr9* during 2005- 2006.Virulence on this gene was discovered in Syria in 2007 (Kassem et al., 2011). Virulence on genes *Lr*9 and *Lr19* in 2015 and 2016 was reported by Walid (2018).

In Bulgaria, during 2015-2016, high efficiency was observed in genes *Lr22A, Lr 22B, Lr28, Lr41, Lr43* and *Lr47*. Good efficiency was observed in genes *Lr2a, Lr2b, Lr25, Lr42* and *Lr46.* Sufficient was the efficiency of genes *Lr24, Lr40*, *Lr48* and *Lr50*. Low efficiency was demonstrated by genes *Lr1, Lr2c, Lr3, Lr3k*a, *Lr10, Lr15, Lr16, Lr17, Lr18, Lr20, Lr21, Lr23, Lr26, Lr29, Lr33, Lr36, Lr38, Lr39, Lr44, Lr45, Lr51, Lr52, Lr60, Lr63, Lr64,* and absolutely inefficient were genes *Lr11*, *Lr26* and *Lr30.*

Conclussion

During the investigated period, 42 phenotypically different pathotypes were identified. Six pathotypes were identified during both years of study. The dominant pathotype in 2015 was PKTTS with 29% distribution, and in 2016 – pathotype TKTTN with 16.6% distribution.

The individual pathotypes were unevenly distributed in the respective agro ecological zones of Bulgaria. The pathotypes with the widest distribution in 2015 – pathotype PKTTS – was found in a greater percent of the samples from Eastern and Central Southern Bulgaria. The most widely distributed pathotype in 2016, pathotype PKTTS, also had uneven distribution in the different agro ecological zones; its percent was higher in samples from North-Eastern and South-Eastern Bulgaria.

The genes for resistance demonstrated variable efficiency. The strong genes *Lr*9 and *Lr19* remained absolutely efficient. Highly efficient during the investigated period were genes *Lr22A, Lr22B, Lr28, Lr41, Lr43* and *Lr47*. Good was the efficiency of genes *Lr2a, Lr2b, Lr25, Lr42* and *Lr46.* The rest of the genes for resistance had sufficient and low efficiency, and genes *Lr11*, *Lr26* and *Lr30* were absolutely inefficient.

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