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Characterization of virulence and diversity of *Puccinia triticina* on wheat in Bulgaria during 2017/2018 growing season

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Abstract

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Leaf rust (*Puccinia triticina* Eriks.) is one of the most widespread diseases on wheat (*Triticum aestivum* L.) in Bulgaria and worldwide. The monitoring researches of the pathogen populations have not only scientific significance, but also practical importance in the breeding for resistance and the distribution of the resistant varieties in the agro cenosis. One hundred and twenty isolates of *Puccinia triticina* were collected from nine agro ecological regions in Bulgaria during vegetative growth seasons 2016/17 and 2017/18. Seventy-nine phenotypically different pathotypes were identified based on the expressed infection type on 20 different varieties (*Lr* genes). Eight of the races (PKTTS, PHTTS, TKTTS, TKTTT, THTTS, THTTD, TKKTJ and PKKTS) identified in the population, were found in previous investigations as well.

Thirty-six new pathotypes were identified in 2017, and in 2018 – 35 new pathotypes. In 2017, with the highest percent of distribution were pathotypes PHKTD and PKTTD (7.9%), and in 2018 – pathotype PKKTS (7.0%). The predominant virulent phenotypes during vegetative growth seasons 2016/17 and 2017/18 were unevenly distributed on the territory of Bulgaria. The genes for resistance varied by their efficiency. During the investigated period, virulence on genes *Lr* 9, *Lr* 19 and *Lr* 43 was not found. Low frequency virulence was determined on genes *Lr* 2b, *Lr* 22B, *Lr* 40, *Lr* 41 and *Lr* 47. Genes *Lr* 1, *Lr* 2c, *Lr* 3, *Lr* 10, *Lr* 11, *Lr* 15, *Lr* 18, *Lr* 20, *Lr* 21, *Lr* 23, *Lr* 26, *Lr* 27+31, *Lr* 29, *Lr* 30, *Lr* 36, *Lr* 37, *Lr* 44, *Lr* 45, *Lr* 51, *Lr* 60, *Lr* 63, *Lr* 64, *Lr* B were with low efficiency during the studied period, while genes *Lr* 14a, *Lr* 16 and *Lr* 17 were completely inefficient.

Keywords: wheat; *Puccinia triticina*; pathotypes; *Lr* genes; efficiency

Introduction

In the recent years, the food security and the stability of the eco-systems are the most alarming themes worldwide. The climatic change is the main reason for the biotic and abiotic types of stress. The climatic changes are related to variations of the annual rainfalls, the mean air temperature, changes of atmospheric CO_2 and the ozone level, the fluctuations of the sea level – all these have their impact on the flora and fauna, as well as on the populations of microorganisms. The pathogens and their populations are also subjected to the changes observed in nature – their number, ratio of genotypes, rate of propagation, rate of mutation process, intensity of genetic exchange (Levitin and Mironenko, 2016). The mutations and migrations in the populations of the pathogens are the main reason for their genetic diversity, and they are inevitably affected by the changes in the conditions of the climate.

Some types of phytopathogenic fungi such as *P. triticina*, the cause agent of leaf rust on wheat, have a wide range of distribution. Among the main diseases on wheat, *P. triticina* is of primary economic importance. The uredospores of the pathogen have several reproductive cycles within a single season. The uredospores are wind-borne over thousands of kilometers and are the most common reason for the occurrence of new races or for their migration from one region to another (Kolmer & Ordonez, 2007).

In order to carry out successful breeding for resistance to the pathogen, information on the efficiency of the individual *Lr* genes and knowledge on the ways in which the variability of the pathogen's virulence affects the new developed varieties are necessary. The genes for virulence are often considered an important criterion for investigating the structure of the population. The changes in it, such as the occurrence of new races, are often challenging to the achieving of durable resistance.

Understanding the genetic variability, the population structure and the connection between the pathotype variation and the genetic diversity in the *P. triticina* population, i.e. the monitoring of the pathogen population, allows the elaboration and introduction of efficient strategies for disease management such as developing resistant varieties.

The aim of this investigation was to present data on the variability and distribution of the pathotypes in the population of *P. triticina* on the territory of Bulgaria, as well as on the variation of the efficiency of the *Lr* genes during the studied period.

Materials and Methods

The investigation was carried out at the Plant Pathology Laboratory of Dobrudzha Agricultural Institute – Bulgaria (DAI) during vegetative growth seasons 2016/2017 – 2017/2018. One hundred and twenty isolates from 42 samples were analyzed (63 isolates from 22 samples in 2017, and 57 isolates from 20 samples in 2018). The analysis on the pathogen's virulence was carried out according to the generally accepted methodology approved for work also at our Plant Pathology Laboratory.

The infection material – leaf fragments with symptoms of *P. triticina* were taken from different wheat varieties during inspection of breeding and production fields at milk-wax maturity stage in 9 agro ecological regions of Bulgaria. The collected samples were dried at room temperature and stored in a desiccator at 4° C until they were subjected to analysis. The monoculture isolates were obtained according to the methodology adopted for work with rusts at the Plant Pathology Laboratory of cereal crops at DAI, which has been described in detail in previous publications (Ivanova, 2012; Ivanova, 2014; Ivanova et al., 2021). For propagation of the pathogen, the universally susceptible cultivar *Michigan amber* was used. For analysis of the virulence, 20 isogenic lines were involved, which were developed on the basis of the susceptible cultivar *Thatcher* and which were grouped in 5 sets.

Based on long-term researches for establishing the virulence of the pathogen, a standard differential set was used until 1997. Over time, the conclusion was made that these differential varieties could not reveal the entire genetic variability, since they contain only 5 genes for resistance (Karzhin & Gospodinova, 1996). Later, a set of 15 isogenic lines was used for race identification, and the race was designated with a digit by the method of Limpert & Muller (1994) in accordance with the modifications in the methodologies adopted in COST 817. Since 2015, our Laboratory uses the North-American nomenclature of Long & Kolmer (1989), amended by Kolmer et al. (2013), which is being applied worldwide.

The isogenic lines used for pathotype differentiation in this research are presented in Table 1, and the sets for race identification according to the manifested infection type produced on the separate differential *Lr* lines are given in Table 2.

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/ Democrt	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 cereal*	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

Results and Discussion

The annual surveys in different agro climatic zones of Bulgaria, which differ by their thermal and moisture supply, allowed collecting infection material from different cultivars with different genetic determination. The samples collected from such varied genetic material were the basis of our monitoring studies on the *P. triticina* population in Bulgaria. The

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	1	2a	2c	3
	Host set 2	9	16	24	26
	Host set 3	3ka	11	17	30
	Host set 4	B	10	14a	18
	Host set 5	3bg	14b	20	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)

analysis of the pathogen population in this study was carried out by the conventional investigations of the pathogen on the virulence and the phenotypic composition of the population. A high level of diversity in the *P. triticina* population was determined during the investigated period on the territory of Bulgaria, which was represented by 79 phenotypically different pathotypes. The distribution of the pathotypes by frequency of occurrence during the period of study is given in Table 3.

Pathotypes PHKTD and PKTTD were with the highest present of occurrence in 2017 (7.9%). Second by occurrence in the 2017 population ranked pathotypes PKKTB and PKKTD (6.3%). Pathotypes PHTTD and MKTTD accounted for 4.8% of the population in 2017. The highest percent of occurrence in 2018 was of pathotype PKKTS (7.0%). A little lower percent (5.3%) was read for pathotypes TKTTS and TKKTS. The rest of the pathotypes in the population were with lower percent of occurrence but they always carry a potential risk of a new race occurrence in the population.

Eight of the races were identified in our previous investigations (Ivanova et al., 2021). Pathotype PKTTS was established in a high percent of the population in 2015 (29%) but its frequency of occurrence in 2016 decreased sharply to 3.3%, and, as evident from the data presented, this pathotype

Table 3. Percentage of *P. triticina* pathotypes identified in Bulgaria during vegetative growth seasons 2016/2017 and 2017/2018

Pathotype	2017 %	2018 %	Pathotype	2017 %	2018 %
BHTSQ	–	1.7	PKKTN	1.6	–
CHKTD	1.6	–	PKKRH	1.6	–
DHKTC	1.6	–	PKTPS	–	3.5
FHKTD	3.2	–	PKKTS	–	7.0
FHTTF	1.6	–	PKKPQ	–	1.7
FKKFP	1.6		PKKTQ	–	1.7
FKKTB	1.6	–	PKTTS	–	1.7
FKHSS	–	1.7	RHRTS	–	1.7
HHTTS	–	1.7	RHTTQ	–	1.7
HKKTS	–	1.7	RKKTL	–	1.7
KHJTJ	–	1.7	RKTTT	–	1.7
MHTTB	1.6	–	TGNTS	–	1.7
MHTTC	1.6	–	TJKTT	–	1.7
MHTTD	1.6	–	THTTC	1.6	–
MHKTD	3.2	–	THTTD	1.6	–
MKKTD	3.2	–	THKTT	–	1.7
MKKTL	1.6	–	THTTS	–	3.5
MKTTD	4.8	–	THKPR	–	1.7
MKTTB	1.6	–	THKPS	–	1.7
MKKTB	1.6	–	THKTS	–	3.5
MKKTC	1.6	–	THTPS	–	1.7
NKKTD	1.6	–	THJTJ	–	1.7
PHDTS	–	1.7	THKPL	–	1.7
PHKTS	–	3.5	THKTQ	–	1.7
PHKTQ	–	1.7	THKSS	–	1.7
PHTTB	3.2	–	TKTTC	1.6	–
PHTTD	4.8	–	TKTTD	3.2	–
PHTTC	3.2	–	TKTTS	–	5.3
PHTPF	1.6	–	TKTTK	–	1.7
PHTTF	1.6	–	TKTTT	–	1.7
PHTTS	–	3.5	TKTTJ	–	1.7
PHKTD	7.9	–	TKTTQ	–	1.7
PHTPC	1.6	–	TKKTS	–	5.3
PKTTB	4.8	–	TKKTJ	–	1.7
PKTTC	1.6	–	TKKTT	–	1.7
PKTTD	7.9	–	TKKPS	–	1.7
PKTTF	1.6	–	TKTPS	–	1.7
PKKTB	6.3	–	TKTPJ	–	1.7
PKKTD	6.3	–	TKSTS	–	1.7
PKKTF	1.6	–			

was not present in samples from 2017; again a low percent of it was present in the 2018 population (1.7%).

Pathotype PKKTS was determined in 2018 with 1.7 % of occurrence, while in 2016 it percent was 3.3%. The observed

tendency was towards lower frequency in the population.

In 2018 pathotype PHTTS was with 3.5% of occurrence; in 2017 it was not identified. The investigations from the previous period showed that in 2015 this pathotype ranked second in the population of the pathogen by frequency of occurrence, and in 2016 its frequency was 10%. For this pathotype, too, a tendency of gradual decrease in the frequency was observed.

Pathotype TKTTS was not found in samples in 2017, but in 2018 its presence amounted to 5.3%. In 2015, the pathotype participated with 7.2% in the population, and in 2016 its distribution was 6.6%. The tendency was towards slight and gradual decrease of the percent of occurrence of this pathotype.

The percent of pathotype TKTTT in 2018 was 1.7%, but it was not found in samples from 2017. In 2015, this pathotype was with 2.9% of occurrence, and in 2016 – with 1.6%. The tendency was also towards a slow decrease, although this pathotype maintained a low percent of occurrence.

Pathotype THTTS was detected with 1.4% frequency of occurrence in 2015, and in 2016 – with 1.6%, but in 2018, its percent increased to 3.5%. The tendency observed in this pathotype was gradual increase of its frequency of occurrence during the period of study.

In 2016 and 2017, the frequency of occurrence remained constant for pathotype THTTD (1.6%). Pathotype TKKTJ was present in the population with 1.6% in 2016, and in 2018 – with 1.7% of occurrence. Significant increase of the frequency of this pathotype was not registered. All other pathotypes, presented in this investigation, have not been established in previous surveys on the territory of Bulgaria. The analysis revealed that in 2017, 36 new phenotypes were identified, and in 2018 – 35.

Such monitoring researches on the pathogen populations are carried out in many places worldwide. Kolmer (2019) reported that in 2017 the *P.triticina* pathotypes most widespread on the territory of USA were MBTNB (11.3%), TFTSB (10.9%) and MCTNB (7%). Using data from research programs and projects (http://www.ars.usda.gov), the *P. triticina* pathotypes predominant for 2018 in different regions of USA were determined and pathotype MNPSD was predominant in the Great Plains with 35% of occurrence. In the regions where common winter red wheat is grown, the predominant pathotype pointed out was MBTNS, and in Minnesota, North and South Dakota, pathotype TBBGS was predominant. The analysis of the population in the North Caucasus region (Volkova et al., 2019) revealed that the predominant races in 2017 and 2018 in the west zone of this region were DCRL and LBLL, and in the north zone – PCQB. The authors pointed out that the diversity index of the population for both years was rather high (0.97 – 0.99). Walid et al. (2019) pointed out two main predominant pathotypes, STTTK (9.81%) and TTTTT (5.1%) on the territory of Egypt in 2017 and 2018.

The climatic conditions in Bulgaria in 2017 and 2018 were favorable for the development of the cereal crops, and also for the development of *P. triticina*. Leaf rust was detected in all surveyed agro ecological zones of Bulgaria. The analysis revealed that the distribution of the pathotypes in the agro ecological zones on the territory of the country was uneven. Table 4 gives the number of virulent isolates from the predominant phenotypes in the surveyed agro ecological zones.

The analysis showed that pathotype PHKTD was in samples from Burgas, Chepintsi, Brushlen and Dobrich, but it was not found in locations from Central and South Bulgaria, nor in locations in North-West and Central North Bulgaria. Pathotype PKTTD was also identified in samples from North-East and South-East Bulgaria in locations Brushlen, Dobrich and Burgas, as well as in a single sample from Central North Bulgaria from location Borovan, but it was not determined in any location in Central South, West or North-

Table 4. Number of virulent isolates during the period of study over agro ecological zones

Location	PHK TD	PKT TD	PKT TB	PKK TD	MKT TD	PKK TS	TKT TS	TKK TS	THT TS	THK TS
	17	17	17	17	17	18	18	18	18	18
Burgas	2	1	0	0	0	0	1	1	0	0
Radnevo	0	0	2	0	0	1	1	0	0	0
Ivailo	0	0	0	1	1	1	1	0	0	0
Chepintsy	1	0	0	1	0	0	0	2	1	0
Svistov	0	0	0	0	0	0	0	0	1	0
Selanovtsy	0	0	1	0	0	1	0	0	0	0
Borovan	0	1	0	1	1	0	0	0	0	0
Brushlen	1	2	0	1	1	1	0	1	0	0
Dobrich	1	1	0	0	0	0	0	1	0	2

West Bulgaria. Pathotype PKTTB was not found in the locations from West, Central North, North-East and South-East Bulgaria. The pathotype was identified only in samples from South Bulgaria (location Radnevo) and North-West Bulgaria (location Selanovtsi). Pathotype PKKTD was identified in samples from South, West, Central North and North-East Bulgaria. It was absent at the other locations of the country. Pathotype MKTTD was found in samples from Central North, Central South and North-East Bulgaria.

This pathotype was not found in samples from the other locations. Pathotype PKKTS was determined in samples from Central South Bulgaria (locations Radnevo and Ivaylo), as well as from North-West and North-East Bulgaria. Pathotype TKTTS was identified in samples from South-East, West and North-East Bulgaria. Pathotype THTTS was found in samples from West Bulgaria (location Chepintsi) and North-West Bulgaria (location Svishtov). It was not identified in the other parts of the country. Pathotype THKTS was identified only in samples from Dobrich and was also absent in the other parts of Bulgaria.

In the monitoring researches, special attention is paid to the efficiency of the genes and how it changes depending on the variation of the races in the population. A number of studies have shown that *P. triticina* often acquires new and aggressive virulence capable of overcoming the specific genes (Pretorious et al., 2007, 2015; Terefe et al., 2011; Boshoff et al., 2018). The long-term growing of cultivars, which carry specific genes for resistance, exercises strong selective pressure on *P.triticina*, which can bring about high diversity in the pathogen's population.

Kolmer (2019) reported that in 2017 higher virulence was observed on genes *Lr* 9, *Lr* 24 and *Lr* 39 in three agro ecological regions. Virulence on *Lr* 26 was found in all agro ecological regions of US, except for one. Virulence on *Lr* 1 was registered in 100% of the investigated areas, and virulence on *Lr* 42 was not detected in none of the regions. He

Table 5. Efficiency of the genes

Lr-genes	2017		2018		Total number	Average %	*Lr*-genes	2017		2018		Total number	Average %
	Avirulent isolates	%	Avirulent isolates	%				Avirulent isolates	%	Avirulent isolates	%		
1	7	11.1	4	7.0	11	9.2	29	13	20.6	22	38.6	35	29.2
2a	58	92.1	17	29.8	75	62.5	30	0	0	5	8.8	5	4.2
2b	54	85.7	44	77.2	98	81.7	32	1	1.6	10	17.5	11	9.2
2c	15	23.8	7	12.3	22	18.3	33	2	3.2	4	7.0	6	5
3	3	4.8	2	3.5	5	4.2	34	2	3.2	8	14.0	10	8.3
3ка	31	49.2	33	57.9	64	53.3	35	0	0	21	36.8	21	17.5
9	63	100	57	100	120	100	36	0	0	5	8.8	5	4.2
10	3	4.8	11	19.3	14	11.7	37	20	31.7	0	0	20	16.7
11	0	0	3	5.3	3	2.5	38	40	63.5	1	1.7	41	34.2
14a	0	0	0	0	0	0	39	45	71.4	9	15.8	54	45
14b	63	100	2	3.5	65	54.2	40	54	85.7	32	56.2	86	71.7
15	1	1.6	2	3.5	3	2.5	41	63	100	56	98.3	119	99.2
16	0	0	0	0	0	0	42	46	73	22	38.6	68	56.7
17	0	0	0	0	0	0	43	63	100	57	100	120	100
18	2	3.2	3	5.3	5	4.2	44	29	46	3	5.3	32	26.7
19	63	100	57	100	120	100	45	21	33.3	1	1.7	22	18.3
20	25	39.7	10	17.5	35	29.2	46	30	47.6	9	15.8	39	32.5
21	0	0	3	5.3	3	2.5	47	63	100	41	72	104	86.7
22A	2	3.2	5	8.8	7	5.8	48	35	55.5	41	72	76	63.3
22B	62	98.4	56	98.2	118	98.3	50	51	81	1	1.7	52	43.3
23	1	1.6	11	19.3	12	10	51	29	46	3	5.3	32	26.7
24	26	41.3	25	43.9	51	42.5	52	42	66.6	3	5.3	45	37.5
25	35	55.5	43	75.4	78	65	60	11	17.5	0	0	11	9.2
26	1	1.6	2	3.5	3	2.5	63	29	46	0	0	29	24.2
27+31	2	3.2	10	17.5	12	10	64	15	23.8	0	0	15	12.5
28	47	74.6	16	28.1	63	52.5	B	0	0	3	5.3	3	2.5

also reported higher virulence on genes *Lr* 2a, *Lr* 11, *Lr* 18 and *Lr* 21 in comparison to 2016. In South Africa, Boshoff et al. (2018) reported increasing frequency of virulence of the new emerging races on the specific genes *Lr* 3, *Lr* 15, *Lr* 20 and *Lr* 26. Volkova et al. (2020) reported that in the North Caucasian region virulence on genes *Lr* 9, *Lr* 42, *Lr* 47 and *Lr* 50 was not found, but single virulent isolates were identified, which had overcome the resistance of genes *Lr* 19, *Lr* 24, *Lr* 29, *Lr* 41, *Lr* 43+24, *Lr* 45 and *Lr* 52. The North Caucasian region has often been described as a risky epiphytotic zone with high variability of the *P. triticina* populations. Genes *Lr* 9, *Lr* 42, *Lr* 47 and *Lr* 50 were reported as absolutely efficient for the North Caucasion population, but in other parts of Russia (Ural, West Siberia and Central Russia), virulent isolates on genes *Lr* 9, *Lr* 19, *Lr* 24, *Lr* 29, *Lr* 41, *Lr* 43+24, *Lr* 45 and *Lr* 52 were found; these genes have lost their efficiency as a result from the new emerging virulent isolates (Gultyaeva et al., 2015).

In Bulgaria, changes in the efficiency of the genes for resistance were also observed over years. Table 5 presents the efficiency of the resistant genes during the investigated vegetative growth periods 2016/2017 and 2017/2018.

The data for the period showed that genes *Lr* 9, *Lr* 19 and *Lr* 43 remained absolutely efficient. Absolute efficiency during the first year of the investigation was demonstrated by gene *Lr* 14b, but in the second year, its efficiency sharply decreased to 3.5%. The same tendency was observed for gene *Lr* 22A, too. Its efficiency in the previous period was high, while in this study low efficiency of this gene was registered. A probable reason can be the altered racial composition and the occurrence of more aggressive pathotypes in comparison to our previous researches. During the first year of the investigation, gene *Lr* 41 also showed 100% efficiency but in the second year single virulent pathotypes occurred, which succeeded in overcoming its resistance to some extent; nevertheless, averaged for the period, its efficiency remained high and close to the absolute (99.2%). With high efficiency during the reported period were genes *Lr* 2b, *Lr* 22B, *Lr* 40, *Lr* 41 and *Lr* 47. In comparison to the preceding period 2015/2016, a decrease in the efficiency from high to good was registered for genes *Lr* 2a, *Lr* 28 and *Lr* 42. Good efficiency was determined for genes *Lr* 2a, *Lr* 3ka, *Lr* 14b, *Lr* 24, *Lr* 25, *Lr* 28, *Lr* 38, *Lr* 39, *Lr* 42, *Lr* 46, *Lr* 48, *Lr* 50 and *Lr* 52. In comparison to the previous vegetative growth period 15/16, the same tendency towards low efficiency was observed in genes *Lr* 1, *Lr* 2c, *Lr* 3, *Lr* 10, *Lr* 11, *Lr* 15, *Lr* 18, *Lr* 20, *Lr* 21, *Lr* 23, *Lr* 26, *Lr* 27+31, *Lr* 29, *Lr* 30, *Lr* 36, *Lr* 37, *Lr* 44, *Lr* 45, *Lr* 51, *Lr* 60, *Lr* 63, *Lr* 64, *Lr* B. Absolutely inefficient during this period were genes *Lr* 14a, *Lr* 16 and *Lr* 17.

Conclusion

During vegetative growth periods 2016/2017 and 2017/2018, the variability in the *P. triticina* population on the territory of Bulgaria was studied. One hundred and twenty monoculture isolates were investigated, and 79 phenotypically different pathotypes were determined. Eight of the pathotypes, PKTTS, PHTTS, TKTTS, TKTTT, THTTS, THTTD, TKKTJ and PKKTS, were identified in the previous vegetative growth period. Thirty-six phenotypically different pathotypes were identified in 2017, and in 2018 – thirty-five new ones.

The dominant pathotypes in the population during 2017 were PHKTD and PKTTD (7.9%), and in 2018, the highest percent of occurrence was determined for pathotype PKKTS (7.0%). The pathogen pathotypes identified in the population were unevenly distributed on the territory of Bulgaria.

The genes for resistance demonstrated variable efficiency. Absolutely efficient during the investigated period were genes *Lr* 9, *Lr* 19 and *Lr* 43. Highly efficient were genes *Lr* 2b, *Lr* 22B, *Lr* 40, *Lr* 41 and *Lr* 47. Genes *Lr* 2a, *Lr* 3ka, *Lr* 14b, *Lr* 24, *Lr* 25, *Lr* 28, *Lr* 38, *Lr* 39, *Lr* 42, *Lr* 46, *Lr* 48, *Lr* 50 and *Lr* 52 were with good efficiency. Low efficiency was demonstrated by genes *Lr* 1, *Lr* 2c, *Lr* 3, *Lr* 10, *Lr* 11, *Lr* 15, *Lr* 18, *Lr* 20, *Lr* 21, *Lr* 23, *Lr* 26, *Lr* 27+31, *Lr* 29, *Lr* 30, *Lr* 36, *Lr* 37, *Lr* 44, *Lr* 45, *Lr* 51, *Lr* 60, *Lr* 63, *Lr* 64, *Lr* B, and absolutely inefficient during this period were genes *Lr* 14a, *Lr* 16 and *Lr* 17.

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