Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2020, Vol. 4 (3), 353-365
https://doi.org/10.29329/ijiaar.2020.274.8


Original article

# Monitoring on the Pathogen Population of *P. triticina* in Bulgaria during 2013-2014

Vanya Ivanova *

Dobrudzha Agricultural Institute – General Toshevo, 9520, Bulgaria

**Abstract**

The population studies on the cause agents of the diseases in agricultural crops are an indispensable stage in the development of a scientifically-coordinated strategy in the breeding for resistance. This investigation follows the changes in the population structure of *P. triticina* during harvest years 2013-2014. The analysis on the population structure was carried out according to the methodology adopted at the Plant Pathology Laboratory of Dobrudzha Agricultural Institute – General Toshevo, Bulgaria. We developed 139 monoracial isolates from 47 samples. A total of 59 pathotypes were identified (27 in 2013 and 32 in 2014), 5 of the pathotypes (02762, 12722, 12762, 12763 and 52762) being determined during the two years of the investigation. Twenty-five new pathotypes were identified, which were not detected during 2000-2012. The distribution of the pathotypes in the individual agro ecological zones was uneven. Three of the pathotypes were dominant: 12762, 52762 and 53763.The genes for resistance to this pathogen demonstrated variable efficiency. The strong genes Lr 9 and Lr 19 were absolutely efficient in 2013, but in 2014 pathotypes were identified, which overcame their resistance. During the first years of the study, genes Lr 2c, Lr 9, Lr 19, Lr 22A, Lr 22B, Lr 25, Lr 41 and Lr 45 reacted as absolutely efficient, while during the second year absolute efficiency was demonstrated by genes Lr 22A, Lr 22B and Lr 25. Good was the efficiency of genes Lr 2a, Lr 2b, Lr 3ka, Lr 24, Lr 42, Lr 44, Lr 45, Lr 46 and Lr 47. Low efficiency was found in genes Lr 1, Lr3, Lr 10, Lr 11, Lr 15, Lr 16, Lr 17, Lr 18, Lr 20, Lr 21, Lr 23, Lr 26, Lr 36, Lr 37, Lr 39, Lr 48, Lr 51 и Lr 52. Absolutely inefficient were genes Lr 30, Lr 38 and Lr 60.

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

**Received:** 19 November 2019 * **Accepted:** 28 September 2020 * **DOI:** https://doi.org/10.29329/ijiaar.2020.274.8

* **Corresponding author:**

Vanya Ivanova, Dobrudzha Agricultural Institute – General Toshevo, 9520, Bulgaria.
Email: vkiryakova@yahoo.com

## INTRODUCTION

Fungal diseases, together with drought, are the main limiting factor for wheat production in Bulgaria and worldwide. Leaf rust caused by *P. triticina* is the most common disease on wheat, which sometimes brings about considerable damage to wheat production. As a result from climatic changes and the fast distribution of *P. triticina* forms adaptive to wormer conditions, fast overcoming of the resistance of the cultivars used in production is observed. The causative agent of leaf rust on wheat *P. triticina* has an active formative process, which leads to the occurence of new pathotypes with different combinations of the genes for virulence. Therefore, it is necessary to annually study the intra-population structure of the pathogen. The monitoring of the pathogen population is applied to many pathosystems and is purposeful research work within the breeding programs for developing of cultivars resistant to a certain disease. The aim is to determine the predominant pathotypes and their frequency of occurrence; all this data can be useful in drawing conclusions about the evolutional processes in the populations. Evolutional forces such as mutation, migration, genetic deviation, breeding and recombination change the genetic frequencies in the population and form its genetic structure (Peever et al., 2000). The monitoring of the pathogen population is highly important for the understanding the efficiency and resilience of the host. The pathogen pathotypes interact with the specific host genotypes and this interaction is based on the "gene for gene" principle, but sometimes results in breakdown of resistance occurs within a very short time (Brown, 1995). The knowledge on the population's genetic structure can be successfully used for management and control of the disease.

The aim of this study was to describe the virulence of the *P. triticina* populations over agro ecological zones during 2013-2014 on the territory of Bulgaria, to identify the predominant pathotypes and to determine the efficiency of the resistance genes for this period.

## MATERIALS and METHODS

The study was carried out at the Plant Pathology Laboratory of Dobrudzha Agricultural Institute – General Toshevo (DAI), Bulgaria, in 2013 and 2014. One hundred thirty-nine monoracial isolates were developed out of 47 samples. The analysis on the pathogen's virulence was performed according to the methodology adopted at the Plant Pathology Laboratory of DAI.

Samples from leaves infected with *P. triticina* were collected from different wheat varieties and lines, as well as from mass crops from eight agro ecological regions in Bulgaria. The samples were stored in paper bags with their date and location of collecting marked. These samples were then left to dry at room temperature and were placed in a desiccator at 4$^{o}$C until the performance of the race analysis. The samples collected in summer were placed in Petri dishes on filter paper sprinkled with water and left for 18-20 hours at room temperature till the sores swelled, then the universally susceptible cultivar was inoculated.

Seven days after inoculation, when the sores were well expressed, 3 single well developed apical sores were selected; each sore was transferred to 7-8 days old seedlings from the universally susceptible cultivar *Michigan amber*, so that three single isolates were obtained from each sample. After inoculation, the plants were sprinkled with water using a sprayer and were covered with a glass insulator. The plants thus inoculated stayed in a moist chamber for 24 hours in dark at 18-22$^{o}$ C. After 24 hours, the plants were transferred to a growth chamber under controlled conditions (25/15$^{o}$C day/night, RH>75%, and additional illumination for elongation of the photoperiod 16/8 h at 30000 lx), which were favorable for the development of the plants and the pathogen. Maleic hydrazide 97% (1g per 3 l of water, application 100 ml per pot) was used to improve sporulation. The inoculation of the susceptible cultivar *Michigan amber* with each isolate was repeated several times until sufficient amount of inoculum was accumulated to infect the differential set. The type of infection was determined 7-8 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 type 3-4 is considered an expression of susceptibility. Fifteen isogenic lines presented in Table 1 were used in the differential set.

**Table 1.** Isogenic lines used for pathotype and genetic differentiation

| Lr genes | Pedigree | Origin | Identification number |
|---|---|---|---|
| Lr 1 | Tc*6/ Centenario | Wheat | RL 6003 |
| Lr 2a | Tc*6/ Webster | Wheat | RL 6016 |
| Lr 2b | Tc*6/ Carina | Wheat | RL 6019 |
| Lr 2c | Tc*6/ Loros | Wheat | RL 6047 |
| Lr 3 | Tc*6/ Democrt | Wheat | RL 6002 |
| Lr 9 | Transfer/Tc*6 | *Aegilops umbellulata* | RL 6010 |
| Lr 11 | Tc*2/ Hussar | Wheat | RL 6053 |
| Lr 15 | Tc*6/ Kenya W 1483 | Wheat | RL 6052 |
| Lr 17 | Klein Lucero/ Tc* 6 | Wheat | RL 6008 |
| Lr 19 | Tc*7/Translocation 4 | *Agropyron elongatum* | RL 6040 |
| Lr 21 | Tc*6/RL5406xRL529 | Wheat | RL 6043 |
| Lr 23 | Lee 310/ Tc*6 | Triticum turgidum var.durum | RL 6012 |
| 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 |

The identification of the 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 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 population of P. triticina is rather variable. The multiple studies on the race composition of the pathogen have demonstrated high levels of pathogen diversity worldwide. Each population has unique phenotypes. The P. triticina populations are quite changeable; on the one hand, this changeability is due to climatic changes and changes occurring in the pathogen itself, and on the other – to the constant regeneration of the wheat gene plasma with new sources of race specific and race non-specific resistance. Annually, different number of virulent isolates are being identified in the different parts of the world and this is directly related to the nomenclature system used in the respective country. In North America and Canada, the nomenclature suggested by от Long and Kolmer (1989) and supplemented by several other authors (Kolmer et al., 2007; Kolmer and Lui, 2000; McVey et al., 2004) is used. The early studies on the P. triticina populations on the American continent began in Kansas (Johnston et al, 1968) and in Indiana (Main and Jackson, 1926). The first registered investigations in Canada are from 1931 (Johnson, 1956; Mc Callum and Seto-Goh, 2006). In Nepal, Bangladesh and Pakistan they have their own nomenclature systems. In India, Australia and Africa a binominal system for race identification is used (Nagarajan, 1983; Park, 1996; Pretorius at al., 1987). On the European continent, the designation of races is done by reading the reaction of 15 isogenic lines based on the triplet set principle of Octal codes (Hanzalova and Bartos, 2006, Goyeau et al., 2006; Manninger, 2006; Martinez et al., 2005; Mantovani et al., 2010; Park and Felsenstein, 1998). In Bulgaria, the investigations on the changes in the populations of *P.triticina* are to be found in a series of publications (Ivanova, V. 2012, 2014, 2017). In this investigation related to determining of the physiological specialization of *P. triticina* in harvest years 2013 and 2014, a total of 59 pathotypes were identified (27 in 2013 and 32 in 2014). Five of the pathotypes (02762, 12722, 12762, 12763 and 52762) were identified during both years of the investigation. Three pathotypes with high frequency of occurrence were found out: 12762, 52762 and 53763. Twenty-five new pathotypes were identified, which were not detected during 2000-2012.

Table 2 presents the distribution of the pathotypes over years. Pathotype 12762 was with the highest percent of distribution in 2013 with frequency of occurrence 16.4%, followed by pathotype 52762 with 15% of distribution and pathotypes 42762 and 52562 with 9.6% frequency of occurrence. The rest of the pathotypes were with frequency of occurrence 1.4% - 5.5%.

**Table 2.** Percent of *P. triticina* pathotypes identified in Bulgaria during 2013 – 2014

| Pathotype | 2013 % | 2014 % | Pathotype | 2013 % | 2014 % |
|---|---|---|---|---|---|
| 02560 | 1.4 | - | 42762 | 9.6 | - |
| 02562 | 1.4 | - | 42763 | 1.4 | - |
| 02762 | 2.7 | 1.5 | 52560 | 5.5 | - |
| 10762 | 1.4 | - | 52562 | 9.6 | - |
| 12560 | 2.7 | - | 52563 | 1.4 | - |
| 12562 | 1.4 | - | 52760 | 5.5 | - |
| 12663 | - | 1.5 | 52762 | 15.0 | 1.5 |
| 12702 | 1.4 | - | 52773 | - | 1.5 |
| 12722 | 1.4 | 3.03 | 53762 | - | 3.1 |
| 12723 | - | 1.5 | 53763 | - | 12.1 |
| 12760 | 1.4 | - | 53773 | - | 1.5 |
| 12762 | 16.4 | 6.1 | 53723 | - | 1.5 |
| 12763 | 2.7 | 9.1 | 62562 | 1.4 | - |
| 12733 | - | 1.5 | 63563 | - | 1.5 |
| 12773 | - | 1.5 | 63776 | - | 1.5 |
| 13762 | - | 1.5 | 71772 | - | 1.5 |
| 13763 | - | 1.5 | 72167 | 1.4 | - |
| 13773 | - | 1.5 | 72560 | 1.4 | - |
| 16362 | - | 1.5 | 72564 | 1.4 | - |
| 22773 | - | 1.5 | 72760 | - | 1.5 |
| 32722 | 1.4 | - | 72763 | - | 1.5 |
| 32762 | - | 1.5 | 72765 | 1.4 | - |
| 32763 | - | 3.1 | 72722 | - | 1.5 |
| 32773 | - | 1.5 | 72767 | 1.4 | - |
| 33573 | - | 1.5 | 73562 | - | 1.5 |
| 33762 | - | 1.5 | 73763 | - | 6.1 |
| 33763 | - | 7.6 | 73772 | - | 3.1 |
| 42562 | 5.5 | - | 73773 | | 1.5 |
| 42760 | 2.7 | - | 73767 | - | 1.5 |
| 73746 | - | 1.5 | | | |

The most common pathotype in 2014 was 53763 with 12.1% distribution, followed by pathotypes 12763 (9.1%) and 33763 (7.6%). Based on literary sources, about 70 new races are annually identified in the USA. Researches show that in 2013-2014 pathotypes MBTNB (19.5%), TBBGS (11.6%) and TCRKG (11.3%) prevailed on the territory of the USA, but they were not identified in all agro ecological zones, similar to the results from our study.

In two successive publications, Kolmer and Hughes (2015, 2016) pointed out that in 2013 and 2014 there was a difference in the virulence potential on the territory of USA, which implied a fast changing dynamic population. In our study, the same tendency of a fast-changing population represented through variations in the virulence potential of the population was determined.

In their investigations, Ordonez et. al. (2010) established a high degree of similarity between the life rust genotypes in South and North America implying European origin of the identified races, although evolution of virulence occurred. Park and Felsenstein (1998) found out that the isolates from Northern France and Southern England had identical and similar phenotypes of virulence.

Levitin and Mironenko (2016) made the assumption that the lief rust population in Caucasus originated from the Asian and European populations. According to these authors, there is a population in the west Asian part of Ural, West Siberia, and North Kazakhstan, which is different from the European one. The reason for all these differences observed in the populations from different parts of the world are random mutations in the pathogen leading to the occurrence of new phenotypes with altered virulence (Kolmer et al., 2011).

No less significant are the migrations since the pathogen's spores can be easily transported on air currents over large distances. An example of a migrating isolate is given by R. Park (2015), who reported a new pathotype in West Australia. Pathotype 104-1,3,4,6,7,8,10,12 + 37 was identified in samples collected from four different locations in the northern region of West Australia. According to the author, this was the third isolate migrating from East to West Australia during the past 25 years. The factors of the environment have a significant effect on the proportion of the genotypes in the population (Levitin and Mironenko, 2016). This explains why in individual agro ecological zones there are certain pathotypes, which are absent in others and why their number varies within a wide range. Table 3 presents the virulent pathotypes predominant during the investigated period over agro ecological zones on the territory of Bulgaria. Pathotype 12762 was identified in four of the agro zones during 2013, but the ratio of the pathotypes in the respective zones was uneven. In 2014, this pathotype was also identified in four agro zones and its distribution was even. Second in distribution over years was pathotype 52762. In 2013, samples from this phenotype were collected from six agro ecological regions, while in another two the phenotype was absent (Burgas and Gorski Izvor). In 2014, this pathotype was identified only in one agro ecological zone (Dobrich). Third in distribution was pathotype 53763. In 2013, it was not found in neither of the zones, but in 2014 it was identified in samples from six agro ecological zones, while in two of the zones it was missing (Radnevo and Brushlen).

**Table 3.** Number of virulent isolates to the virulent phenotypes predominant over 8 agro ecological zones.

| Location | 12762 | | 52762 | | 53763 | |
|---|---|---|---|---|---|---|
| | 13 | 14 | 13 | 14 | 13 | 14 |
| Burgas | 0 | 1 | 0 | 0 | 0 | 1 |
| Radnevo | 0 | 1 | 3 | 0 | 0 | 0 |
| Gorsky Izvor | 0 | 1 | 0 | 0 | 0 | 1 |
| Ivailo | 0 | 0 | 2 | 0 | 0 | 1 |
| Chepintsy | 3 | 0 | 1 | 0 | 0 | 2 |
| Selanovtsy | 6 | 0 | 2 | 0 | 0 | 2 |
| Brushlen | 2 | 0 | 1 | 0 | 0 | 0 |
| Dobrich | 1 | 1 | 2 | 1 | 0 | 1 |

Table 4 presents the virulence exhibited on genes determining resistance to *P.triticina* during 2013-2014. In 2013, virulence for genes Lr 9, Lr 19, Lr 22A, Lr 22B, Lr 25, Lr 41 and Lr 43 was not established. In 2014, virulence for genes Lr 3bg, Lr 14b, Lr 22A, Lr 22B, Lr 25 was not found. Virulence below 10 % was determined for genes Lr 2a, Lr 2c, Lr 24, Lr 28 and Lr 47 in 2013, and for genes Lr 9, Lr 28, Lr 41 и Lr 43 in 2014. Virulence higher than 10 % and lower than 50 % during the first year of the testing was determined for genes Lr 3ka, Lr 40, Lr 42, Lr 44 and Lr 45, while during the second year such virulence was demonstrated by genes Lr 2a, Lr 2b, Lr 3ka, Lr 19, Lr 42 and Lr 45. Genes Lr 1, Lr2b, Lr3, Lr 10, Lr 11, Lr 15, Lr 16, Lr17, Lr 18, Lr 20, Lr 21, Lr 23, Lr 26, Lr 27+31, Lr 30, Lr 36, Lr 37, Lr 38, Lr 39, Lr 46, Lr 48, Lr 51, Lr 52, Lr 60 demonstrated virulence higher than 50 % in 2013, and in 2014, a tendency toward higher virulence was found in genes Lr 1, Lr 2c , Lr 3, Lr 10, Lr 11, Lr 15, Lr 16, Lr 17, Lr 18, Lr 20, Lr 21, Lr 23, Lr 26, Lr 27+31, Lr 30, Lr 36, Lr 37, Lr 38, Lr 39, Lr 46, Lr 47, Lr 48, Lr 50, Lr 51, Lr 52 and Lr 60.

The investigations of McCallum and Seto - Goh (2008, 2009) for the territory of Canada showed higher virulence to genes Lr 9, Lr 17a, Lr 24 and Lr 14a, while in genes Lr 2a, Lr 2c and Lr 16 a decrease in virulence was observed. In this part of the world, virulence for genes Lr 19, Lr 21, Lr 29 and Lr 32 was not found. Virulence to Lr 21 in Canada was first established in 2011 (5.2 %), and in 2012 this percent increased to 7.7 %. Under the conditions of Bulgaria, the virulence to Lr 21 was over 50 % (Table 4).

**Table 4.** Virulence on genes determining resistance to *P.triticina* during 2013-2014

| | No attacked LR genes | Virulence < 10% | Virulence > 10% и < 50% | Virulence > 50% |
|---|---|---|---|---|
| 2013 | LR 9, LR 19, LR 22A, Lr 22B, LR 25, LR 41, LR 43 | LR 2a, LR2c, LR 24, LR 28, LR 47 | LR 3ка, LR 40, LR 42, LR 44, LR 45 | LR 1, LR 2в, LR 3, LR 10, LR 11, LR 15, LR 16, LR 17, LR 18, LR 20, LR 21, LR23, LR 26, LR 27+31, LR 30, LR 36, LR 37, LR 38, LR 39, LR 46, LR 48, LR 51, LR 52, LR 60 |
| 2014 | Lr 3bg, Lr 14b, LR 22A, L22B, LR 25 | Lr 9, Lr 28, Lr 41, Lr 43 | Lr 2a, Lr 2b,Lr 3ka, Lr 19,Lr 42, Lr 45 | Lr 1, Lr 2c, Lr 3, Lr 10, Lr 11,Lr15, Lr 16, Lr17,Lr 18, Lr 20, Lr 21, L23, Lr 24, Lr 26, Lr 27+31, Lr 29, Lr 30, Lr 32, Lr 33, Lr 34, Lr 35, Lr 36, Lr 37, Lr 38, Lr 39, Lr 40, Lr 44, Lr 46, Lr 47, Lr 48, Lr 50, Lr 51, Lr 52, Lr 60 |

The results from the analysis on the virulence in the different parts of the world showed differences and variation for the separate genes. Kolmer and Hughes (2016) reported that virulence for genes Lr 16 and Lr 24 was found in none of the agro ecological zones on the territory of the USA in 2014, while virulence to Lr 21 was found in three regions. On the European continent, the investigations of Hanzalova et al. (2017) during 2012-2015 for the territory of the Czech Republic showed that there was no virulence found for genes Lr 9 and Lr 19, and in genes Lr 2a, Lr 2b, Lr 2c, Lr 24 and Lr 28, low frequency virulence was registered. High frequency virulence was registered to genes Lr 26 (79%) and Lr 1 (86%), and for the rest of the genes, the virulence was with frequency 90-100%. For the territory of Slovakia, Hanzalova (2016) reported absolute efficiency of genes Lr 9 and Lr 19, and low virulence to Lr 24. Most of the isolates were virulent to Lr 1, Lr 2a, Lr 2b, Lr 2c, Lr 3a, Lr 10, Lr 11, Lr 13, Lr 15, Lr 17, Lr 21, Lr 23, Lr 26, Lr 28. In Poland, Czajowski et al, (2016) provided data on low frequency virulence to genes Lr 2b, Lr 2c, Lr 23, Lr 28, Lr 38 and Lr 52, and no virulence to genes Lr 2a, Lr 9, Lr 19 and Lr 25. The investigations carried out by Babayants et al. (2015) showed that during 2013 in Ukraine the virulence frequency was high to genes Lr 2a and Lr 2c, and low to genes Lr 24 and Lr 26.In South Asia, according to data by Prasad et al. (2017), the predominant pathotypes were with low virulence frequency to genes Lr 9, Lr 10, Lr 19, Lr24, Lr 28 and with high virulence frequency to genes Lr 13, Lr 14a, Lr 18. The investigations carried out in India, Bangladesh and Nepal (Bhardwaj et al., 2016) pointed out that the predominant pathotypes in these regions were with virulence to genes Lr 1, Lr 3, Lr 10, Lr 11, Lr 12, Lr 13, Lr 14a, Lr 16, Lr 17, Lr 23 and Lr 26, and no virulence was found to genes Lr 9, Lr 24, Lr 25, Lr 32, Lr 39 and Lr 45 during the past 5 years.

There are reports from many parts of the world about overcoming of the resistance of the two strongest genes Lr 9 and Lr 19. Data on the overcoming of the resistance of Lr 9 from USA, and later from Great Britain, was provided by Kolmer et al. (2007). Babayants et al. (2016) reported that the

frequency of the virulent isolates to Lr 9 increased with 13% in 2013 on the territory of Ukraine. Increased frequency of virulence to Lr 9 in the USA and Canada was confirmed by several authors (McCallum et al., 2011; Kolmer et al., 2012; Soliman et al., 2012).

Sibikeev et al. (1996) identified a new pathotype of rust virulent to Lr 19 in Russia. The first report for overcoming of the resistance of this gene in India was in 2004 (Bhardwaj et al., 2005). Our investigation also confirmed the occurrence of pathotypes overcoming the resistance of the strong genes in 2014.

When the frequency of the virulence is higher than 10 % and the aggressiveness of the pathogen increases, the resistant gene can be overcome for a short period. A large part of the Lr genes lose their efficiency and cannot be used as donors of resistance. This imposes the necessity to annually read of the efficiency of the genes for resistance.

The efficiency of the genes in Bulgaria for 2013 and 2014 is presented in Table 5.

**Table 5.** Efficiency on the genes of resistance in Bulgaria during 2013-2014

| Lr genes | 2013 | | 2014 | | Total number of isolates | Average % |
|---|---|---|---|---|---|---|
| | Avirulence isolates | % | Avirulence isolates | % | | |
| 1 | 18 | 24.6 | 5 | 7.6 | 23 | 16.5 |
| 2a | 66 | 90.4 | 38 | 57.6 | 104 | 74.8 |
| 2в | 26 | 35.6 | 35 | 53.0 | 61 | 43.8 |
| 2c | 73 | 100 | 28 | 42.4 | 101 | 72.7 |
| 3 | 1 | 1.4 | 2 | 3.0 | 3 | 2.2 |
| 3ка | 65 | 89.0 | 41 | 62.1 | 106 | 76.3 |
| 9 | 73 | 100 | 65 | 98.5 | 138 | 99.3 |
| 10 | 10 | 13.7 | 0 | 0 | 10 | 7.2 |
| 11 | 0 | 0 | 1 | 1.5 | 1 | 0.7 |
| 15 | 25 | 34.2 | 3 | 4.5 | 28 | 20.1 |
| 16 | 1 | 1.4 | 0 | 0 | 1 | 0.7 |
| 17 | 1 | 1.4 | 2 | 3.0 | 3 | 2.2 |
| 18 | 0 | 0 | 1 | 1.5 | 1 | 0.7 |
| 19 | 73 | 100 | 52 | 78.8 | 125 | 89.9 |
| 20 | 19 | 26.0 | 23 | 34.8 | 42 | 30.2 |
| 21 | 1 | 1.4 | 0 | 0 | 1 | 0.7 |
| 22 A | 73 | 100 | 66 | 100 | 139 | 100 |
| 22 B | 73 | 100 | 66 | 100 | 139 | 100 |
| 23 | 2 | 2.7 | 7 | 10.6 | 9 | 6.5 |
| 24 | 66 | 90.4 | 30 | 45.5 | 96 | 69.1 |
| 25 | 73 | 100 | 66 | 100 | 139 | 100 |
| 26 | 16 | 21.9 | 1 | 1.5 | 17 | 12.2 |
| 27+31 | 35 | 47.9 | 21 | 31.8 | 56 | 40.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 28 | 68 | 93.2 | 63 | 95.5 | 131 | 94.2 |
| 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | 1 | 1.4 | 1 | 1.5 | 2 | 1.4 |
| 37 | 5 | 6.8 | 5 | 7.6 | 10 | 7.2 |
| 38 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | 5 | 6.8 | 0 | 0 | 5 | 3.6 |
| 40 | 55 | 75.3 | 19 | 28.8 | 74 | 53.2 |
| 41 | 73 | 100 | 59 | 89.4 | 132 | 95.0 |
| 42 | 59 | 80.8 | 39 | 59.0 | 98 | 70.5 |
| 43 | 73 | 100 | 64 | 97.0 | 137 | 98.6 |
| 44 | 42 | 57.5 | 1 | 1.5 | 43 | 30.9 |
| 45 | 54 | 73.9 | 37 | 56.0 | 91 | 65.5 |
| 46 | 30 | 41.0 | 14 | 21.2 | 44 | 31.6 |
| 47 | 66 | 90.4 | 29 | 43.9 | 95 | 68.3 |
| 48 | 15 | 20.5 | 5 | 7.5 | 20 | 14.4 |
| 51 | 21 | 28.7 | 2 | 3.0 | 23 | 16.5 |
| 52 | 14 | 19.2 | 11 | 16.3 | 25 | 18.0 |
| 60 | 0 | 0 | 0 | 0 | 0 | 0 |

In 2013, absolute efficiency was determined for genes Lr 2c, Lr 9, Lr 19, Lr 22A, Lr 22B, Lr 25, Lr 41 and Lr 43. In 2014, absolutely efficient were genes Lr 22A, Lr 22B and Lr 25. During the first year, high efficiency of genes Lr 2a, Lr 3ka, Lr 24, Lr 28, Lr 40, Lr 42, Lr 45 and Lr 47 was established.

During the second year, high efficiency was registered in genes Lr 9, Lr 19, Lr 28, Lr 41 and Lr 43. In 2013, good efficiency was registered in genes Lr 44 и Lr 46. In 2014, good was the efficiency of genes Lr 2a, Lr 2b, Lr 3ka, Lr 24, Lr 42, Lr 45 and Lr 47. Low efficiency was detected in genes Lr 1, Lr 3, Lr 10, Lr 15, Lr 16, Lr 17, Lr 18, Lr20, Lr 21, Lr 23, Lr 26, Lr 36, Lr 37, Lr 39, Lr 48, Lr 51 and Lr 52.

Absolutely inefficient were genes Lr 11 and Lr 18 in 2013, and Lr 10, Lr 16, Lr 21 and Lr 39 in 2014. During the two years of testing, genes Lr 30, Lr 38 and Lr 60 demonstrated absolute inefficiency.

**Conclusion**

As a result from the investigation carried out, we established that the population of *P.triticina* during the investigated period of harvest years 2013-2014 was comprised of a large number of pathotypes, which differed by their virulence. The dominant pathotypes were three: 12762, 52762 and 53763. The rest of the pathotypes were with low frequency of occurrence implying large genetic diversity.

Twenty-five new pathotypes were identified, which were not detected during 2000-2012.

The distribution of pathotypes over agro ecological zones was uneven.

The strong genes Lr 9 and Lr 19 remained absolutely efficient in 2013, but in 2014 pathotypes were identified, which had overcome their resistance.

Genes Lr 2c, Lr 9, Lr 19, Lr 22A, Lr 22B, Lr 25, Lr 41 and Lr 45 reacted as absolutely efficient during the first year, and during the second year, genes Lr 22A, Lr 22B and Lr 25 demonstrated absolute efficiency. Good was the efficiency of genes Lr 2a, Lr 2b, Lr 3ka, Lr 24, Lr 42, Lr 44, Lr 45, Lr 46 and Lr 47. Low efficiency was registered in genes Lr 1, Lr 3, Lr 10, Lr 15, Lr 16, Lr 17, Lr 18, Lr20, Lr 21, Lr 23, Lr 26, Lr 36, Lr 37, Lr 39, Lr 48, Lr 51 and Lr 52. Absolutely inefficient were genes Lr 30, Lr 38 and Lr 60.

## REFERENCES

Babayants, L. Babayants, A. Gorash, V. Traskovetskaya, A. Galaeva (2015). Physiologic specialization of *Puccinia triticina* Erikss. and effectiveness of Lr-genes in the south of Ukraine during 2013–2014 Chilean Journal of Agricultural research, 75, 4, pp. 443–450.

Bhardwaj S. C., M. Prashar, S. Kumar, S. K. Jain, D. Datta (2005). Lr 19 resistance in wheat becomes susceptible to *Puccinia triticina* in India, Plant Dis. 89, 12, pp. 1360.

Bhardwaj S. C., M. Prashar, S. Kumar, O. P. Gangwar, N. Gupta, P. Prasad, H. Khan (2016). Patterns of physiologic diversity of *Puccinia triticina* on wheat in Indian subcontinent during 2008-2013. Indian Journal of Agricultural Sciences 86, 1, pp. 55-64.

Brown J. K. M, (1995). Pathogen responses to the management of disease resistance genes. Advances in Plant Pathology 11, pp. 75-102.

Czajowski G., P. Czembor, M. Radecka-Janusik (2016). Virulence of *Puccinia triticina* the causal agents of wheat leaf rust in Poland in the years 2013-2015. Biuletyn Instytutu Hodowli I Aklimatyzacji Roslin , pp. 13-21.

Goyeau H., Park R., Schaeffer B., Lannou C. (2006). Distribution of pathotypes with regard to host cultivars in French wheat leaf rust populations. Phytopathology 96, pp. 264–273.

Hanzalova A., Bartos P. (2006). Physiologic specialization of wheat leaf rust (*Puccinia triticina* Eriks.) in the Czech Republic in 2001-2004. Czech J Genet Plant Breed 42, pp. 126–131.

Hanzalova A., P. Bartos, T. Sumikova (2016). Virulence of Wheat Leaf Rust (*Puccinia triticina* Eriks.) in the Years 2013-2015 and Resistance of Wheat Cultivars in Slovakia. Cereal Research Communications 44, 4, pp. 585-593.

Hanzalova A., P. Bartos, T. Sumikova (2017). Pathotypes of wheat leaf rust (*Puccinia triticina* Eriks.) and resistance of registered cultivars in the Czech Republic in 2012-2015. Czech. J. Genet. Plant Breed.,53, 3, pp. 122-126.

Ivanova V. (2012). Studies on resistance to common wheat and other species to the cause of brown rust *Puccinia triticina* Eriks. Ph.D tesisis DAI – General Toshevo (in Bg ).

Ivanova V. (2014). Race and Virulence Dynamics of *Puccinia triticina* and Effectiveness of Lr genes in Bulgaria During 2005-2009. Turkish J. of Agricultural and Natural Sciences, Special Issue 1, pp. 709-720.

Ivanova V. (2017). Physiologic Specialization of Wheat Leaf Rust (*Puccinia triticina* Eriks.) in Bulgaria in 2010-2012 3rd International Symposium for Agriculture and Food, Ohrid, Republic of Macedonia, 18-20 October 2017 ( in press )

Johnson, T. (1956). Physiologic races of leaf rust of wheat in Canada 1931 to 1955. Can. J. Agric. Sci. 36, pp. 323-332.

Johnston, C. O., Caldwell, R. M., Compton, L. E., and Browder, L. E. (1968). Physiologic races of *Puccinia recondita* f. sp. *tritici* in the United States from 1926 through 1960. USDA Technical Bulletin pp. 1393, U.S. Department of Agriculture, Washington, DC.

Kolmer J. A, Liu J. Q. (2000). Virulence and molecular polymorphism in international collections of the wheat leaf rust fungus *Puccinia triticina*. Phytopathology 90, pp. 427–436.

Kolmer J. A., Jin Y., Long D. L. (2007). Leaf and Stem Rust of Wheat in the United States. Aust. J. Agric. Res. 58, pp. 631-638.

Kolmer J. A., M. E. Ordonez, M. E. Manisterski, Y. Anikster (2011). Genetic Differentiation of *Puccinia triticina* Populations in the Middle East and Genetic Similarity with Populations in Central Asia . Phytopathology, 101, N7, pp. 870-877.

Kolmer J. A., D. L. Long and M. E. Hughes (2012). Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2010. Plant Disease 96, pp. 1216-1221.

Kolmer J. A. and Hughes, M. E. (2015). Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2013. Plant Dis. 99, pp. 1261-1267.

Kolmer, J. A. and Hughes, M. E. (2016). Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2014. Plant Dis. 100, pp. 1768-1773.

Limpert E. and Muller K. (1994). Designation of Pathotypes of Plant Pathogens, J. Phtopathology 140, pp. 346-358.

Long D. L, Kolmer J. A (1989). A North American system of nomenclature for *Puccinia recondita* f. sp. *tritic*i. Phytopathology 79, pp. 525-529.

Levitin M. M., N. V. Mironenko, (2016). Structure and ranges of phytopathogenic fungi populations. Interdisciplinary Scientific and Applied Journal Biosphere , 8, No. 2, pp. 216-225 (Ru).

Mains, E. B. and Jackson, H. S. (1926). Physiological specialization in the leaf rust of wheat, *Puccinia triticina*. Phytopathology 16, pp. 89-120.

McCallum, B. D., Seto-Goh, P. (2006). Physiological specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2004. Can. J. Plant Pathol. 28, pp. 566-576.

McCallum, B. D., Seto-Goh, P. (2008). Physiological specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2005. Can. J. Plant Pathol. 30, pp. 124-132.

McCallum, B. D., Seto-Goh, P. (2009). Physiological specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2006. Can. J. Plant Pathol. 31, pp. 80-87.

McCallum, B. D., Seto-Goh, P., A. Xue. (2011). Physiological specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2008. Can. J. Plant Pathol. 33, pp. 541-549.

Manninger K. (2006). Physiological specialization of *Puccinia triticina* on wheat and triticale in Hungary in 2004. Acta Phytopathol. Entomol. Hung. 41, pp. 93-100.

Mantovani P., Maccaferri M., Tuberosa R., Kolmer J. A. (2010). Virulence phenotypes and molecular genotypes in collections of *Puccinia triticina* from Italy. Plant Dis. 94, pp. 420-424.

Martinez F., Sillero J. C., Rubiales D. (2005). Pathogenic specialization of *Puccinia triticina* in Andalusia from 1998 to 2000. J Phytopathol. 153, pp. 344–349.

McVey D. V., Nazim M, Leonard K. J., Long D. L. (2004). Patterns of virulence diversity in *Puccinia triticina* on wheat in Egypt and the United States in 1998-2000. Plant Dis. 88, pp. 271-279.

Nagarajan S., Nayar S. K. and Bahadur P. (1983). The proposed brown rust of wheat *Puccinia recondita* f. sp. *tritici* virulence monitoring system. Current Science 52, pp. 413-416.

Ordon˜ez M. E, Germa´n S. E, Kolmer J. A. (2010). Genetic differentiation within the *Puccinia triticina* population in South America and comparison with the North American population suggests common ancestry and intercontinental migration. Phytopathology 100, pp. 376-383.

Park R. F. (1996). Pathogenic specialization of *Puccinia recondita* f. sp. *tritici* in Australia and New Zealand in 1990 and 1991. Austr. Plant Pathol. 25, pp. 12-17.

Park R F., Felsenstein F. G. (1998). Physiological specialization and pathotype distribution of *Puccinia recondita* in Western Europe, 1995. Plant Pathol. 47, pp. 157-164.

Park R. F. (2015). Long term surveys of pathogen populations underpin sustained control of the rust diseases of wheat in Australia. Journal and Proceedings of the Royal society of New South Wales. Vol. 148, Issue 455/456, pp. 15-27.

Peever T .L., Zeigler, T. S. Dorrance, A. E., Correa Victoria, F. S., Martin, S. (2000). Pathogen Population Genetics and Breeding for Disease Resistance. Doi:10.1094/APS net Feature.

Prasad P., S. C. Bhardwaj, O. P. Gangwar, S. Kumar, H. Khan, Sh. Kumar, H. C. Rawal, T. R. Sharma (2017). Population differentiation of wheat leaf rust fungus *Puccinia triticina* in South Asia. Current Science, 112, N 10, pp. 2073-2084.

Pretorius Z. A., Rijkenberg F. H. J., Wilcoxson R. D. (1987). Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa from 1983 through 1985. Plant Dis 71, pp. 1133-1137.

Sibikeev S. N., V. A. Krupnov, S. A. Voronina, V. A. Elesin (1996). First report of leaf rust pathotypes virulent to highly effective Lr-genes transferred from Agropyron species to bread wheat, Plant Breed. 115, pp. 276-278.

Soliman N. E. K., Abdelbacki A. M. M, Najeeb M. A. A, Omara R. I. (.2012). Geographical distribution of physiological races of *Puccinia triticina* and postulation of resistance genes in new wheat cultivars in Egypt. ESCI J. Plant Pathol. 1, pp. 73-80.

Stakman, E. C., D. M. Stewart and W. Q. Loegering (1962). Identification of physiological races of *Puccinia graminis var. tritici.* Agric. Res. Serv. E617 (United States Department of Agriculture: Washington DC).