Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2019, Vol. 3 (3), 442-454
https://doi.org/10.29329/ijiaar.2019.206.10

Original article

Occurrence and Relative Prevalence of Fungal Pathogens on Durum Wheat [1]

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Abstract

An assessment of six Bulgarian and six foreign varieties of durum wheat was carried out for the occurrence of foliar diseases caused by fungal pathogens on a natural background. Field experiments were performed at two locations (Chirpan and Sofia) situated in different climatic zones of Bulgaria for six successive growing seasons (2012 – 2017) with exception of 2015 in Chirpan. The results of this research showed that the population of foliar pathogens of durum wheat was heterogeneous and included 13 fungal species. Some differences in symptom severity and relative proportion of the pathogens among varieties and years and between locations were found. Of the leaf spotting fungi, the causal agent of tan spot (*Pyrenophora tritici-repentis*) was the most prevalent in both prospected areas. The fungi belonging to Septoria leaf blotch complex (*Parastagonospora avenae* f. sp. *triticea*, Pa. *nodorum* and *Zymoseptoria tritici*) and newly found species *Phaeophleospora* sp. were more frequently isolated from leaf samples taken in Sofia location. *Monographella nivalis* and *Cladosporium herbarum* had contribution to the leaf spotting in both surveyed locations in some of the studied years. *Cochliobolus sativus* occurred only occasionally. Of the three rust fungi, Puccinia striiformis f. sp. striiformis was the predominant species. The first record of yellow rust was made in 2013. It was the most common disease in both localities in 2014 and 2016 due to warmer winters, which favored the survival of YR. The race analysis performed in Global Rust Reference Center revealed the appearance and distribution of a new race (Warrior) in Bulgaria. The weather conditions were exceptionally favourable for the development of certain diseases in some years. In this relation the influence of some elements of the climate on the pathogen prevalence was discussed. Except the weather other factors like cultivar characteristics, inoculum level, soil type, and predecessor played a role in changing spectrum of phytopathogenic fungi. In common the level of fungal diseases in Sofia region was higher compared to Chirpan. The studied varieties differed in their susceptibility to fungal diseases.

Keywords: Leaf diseases, Septoria leaf blotch, Rust, Phytopathogenic fungi, Weather conditions.

Received: 13 September 2018 * **Accepted:** 10 July 2019 * **DOI:** https://doi.org/10.29329/ijiaar.2019.206.10

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[1] A part of this study was presented at the International Agricultural, Biological and Life Science Conference, Edirne, Turkey, September 2-5, 2018.

INTRODUCTION

Durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husnot.) occupies about 17 million ha, which represents about 8% of total area under wheat in the world (Petrova, 2005). It is grown mainly in the Mediterranean region including North Africa, South Europa and West Asia. Annually, global production amounts to 37.5 million tons, which is 5% of the total wheat production. The European Union is the biggest producer of durum wheat with more than 10.0 million tons of grain, with Italy playing a leading role: 50% of the durum area and production (Porceddu and Blanco, 2014). Biotic and environmental stresses significantly limit the crop yield and downgrade the commercial and utilization value of harvested grain. Globally changing climate could worsen these constraints (Juroszek and von Tiedemann, 2013). It may provoke changes in pathogen occurrence, an increased impact of already present pathogens, and introduction of new or emerging species due to global trade and travel and extreme weather phenomena. If suitable conditions exist, establishment and long-term survival of such pathogens could occur (Roos et al., 2011). Some diseases favored by cool temperatures may become less important, whereas warm-temperature diseases may increase in future years. Changes in disease occurrence will impact disease management practices. It is important to focus on early detection of new disease problems in a crop growing area.

Wheat diseases are caused by a wide range of organisms. Fungal pathogens are responsible for 15-20% of the annual losses (Figueroa et al., 2018). Rusts, powdery mildew and leaf spotting complex are among the economically most significant fungal diseases. They have great impact on wheat production worldwide and provoke increased efforts in wheat breeding for combined disease resistance (Ruzgas et al., 2002; Šíp et al., 2005; Ali et al., 2008; Figueroa et al., 2018).

Durum wheat is a traditional crop for Bulgaria and the breeding program has considerable achievements (Dechev et al., 2010). In recent years, and especially after Bulgaria's accession to the EU, free import of seeds of foreign varieties has begun, which requires their evaluation in the conditions of our country.

The purpose of present investigation was to carry out an assessment of Bulgarian and foreign durum wheat varieties for the occurrence of foliar diseases caused by fungal pathogens on a natural background.

Material and Methods

Plant material, field experiments and pathogen identification

In 2010 and 2011 preliminary observations on the occurrence and development of foliar diseases caused by fungal pathogens were performed at the experimental fields of Institute of Plant Physiology and Genetics (IPPG) - Sofia and Field Crops Institute (FCI) - Chirpan. Commercial fields of durum wheat in eight different agro-ecological regions were also visited. In the period from 2012 to 2017 field

experiments were carried out at two locations (Sofia and Chirpan) with an exception of 2015 in Chirpan. Twelve winter varieties of durum wheat were involved in the investigation. The six Bulgarian varieties (Progress, Vazhod, Victoria, Predel, Zvezditsa and Deyana) have been created at the FCI – Chirpan and are commonly grown in Bulgaria. The six foreign varieties originate from different European countries: Saragolla and Meridiano (Italy), Auradur (Austria), Pescadou (France), Yukon (Germany) and GK Bétadur (Hungary). The trials were arranged in a randomized block design with two replicates which consisted of 3 rows 2 m long of each cultivar.

Leaf samples were collected in June when the crop was approaching maturity. Ten leaves (usually flag leaves) of each cultivar were taken randomly from each of both replicates. Only the leaves bearing lesions bigger than 1 cm^2 were used for further phytopathological analysis. After visual assessment of the size and shape of the spots an observation of the pathogen reproductive structures was made under light microscope. The fungal identification was made on the basis of a set of morphological and cultural features with the highest taxonomic value.

Yellow rust infected leaf samples were collected in May 2015 and 2016 and sent to Global Rust Reference Center (GRRC) in Denmark for race analysis.

Statistical analysis

The data were statistically processed using the software Statgraphics Centurion XVII. Analysis of variance, calculation of the least significant difference and Tukey-Kramer multiple comparison test was performed at $P \leq 0.05$.

Meteorological data

Sofia and Chirpan are situated in two different climatic zones of Bulgaria: Moderate continental and Transitory continental, respectively.

Moderate continental zone includes two regions: the Climatic region of the Danubian Plain and the Climatic region of high fields in West Central Bulgaria. Sofia (alt. 539 m) is situated in the latter one. It is characterized by the relief of the terrain, the higher altitude and resulting climatic features, which affect the temperature conditions in winter. Although the region is situated to the south of the Balkan Mountains, the winter here is harsher than the one in the Danubian Plain. Winter rainfalls in the region are smaller compared to those in the Danubian plain and are about 80-120 mm. However, the snow cover is more resistant due to lower air temperatures. The average daily air temperature reach up to 5°C in late March and early April, and above 10°C in early May. Spring rainfalls are relatively larger than the winter ones (145-175 mm). The summer in this region is cooler compared to that in the Danubian Plain. Maximum air temperatures in this region reach up to 32-34°C. The average monthly air temperature in the warmest month is 19-21°C. Summer rainfall is usually between 170-210 mm. Their significant quantity and relatively lower temperatures characterize it as a region with weaker summer

droughts than in the Danubian Plain. Autumn is a little warmer than spring. The average air temperature drops below 10°C. The amount of precipitation in autumn is between 125-175 mm. The annual distribution of the rainfalls has an expressed continental character, similar to the one in the Danubian Plain.

Chirpan (alt. 168 m) is situated in the Climatic region of Eastern Central Bulgaria of Transitory continental zone. It is characterized by relatively mild winters (average air temperature January is around 0°C). The snow cover maintains for a relatively short period of time than in northern Bulgaria – 20-30 days. Rainfalls are about 100-150 mm, but only 30-35% of snow. Spring comes relatively early. It is a bit cooler than the autumn. At the end of February to the beginning of March the average air temperature steadily rises above 5°C and towards the end of November and the beginning of December is reduced permanently under 5°C. Summer is hot. Rainfall conditions in this region have a continental character with minimum in winter (February) and maximum in summer (June).

Meteorological data were collected on site at both locations with special emphasis to the major climate factors, temperature and humidity. The deviation of monthly average temperature from climatic norm for the period 1981-2010 in Sofia (Fig. 1) and Chirpan (Fig. 2) and the deviation of monthly rainfall sum for both locations (Fig. 3 and 4) is presented below.

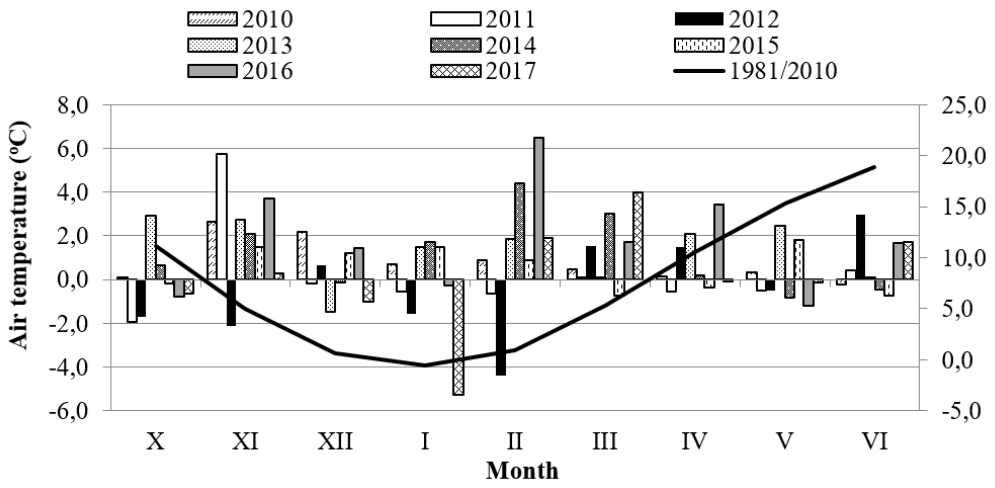


Figure 1. Deviation of monthly average air temperature from climatic norm for the period 1981-2010 and climatic norm (1981-2010) in Sofia

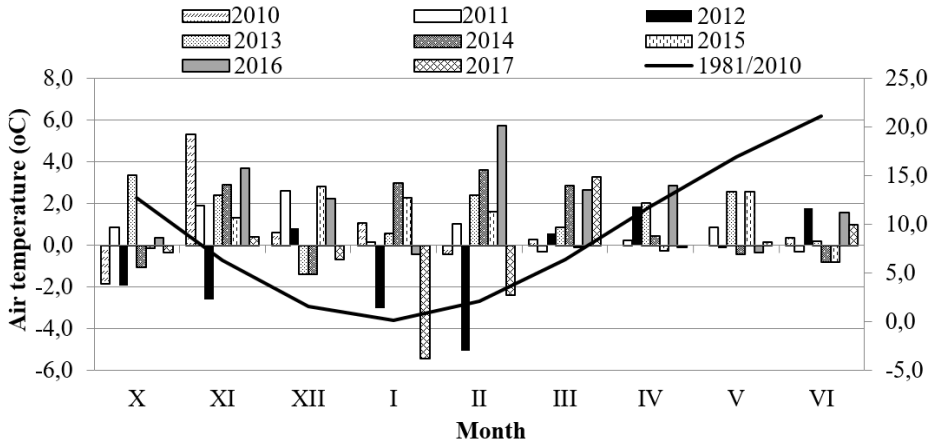


Figure 2. Deviation of monthly average air temperature from climatic norm for the period 1981-2010 and climatic norm (1981-2010) in Chirpan

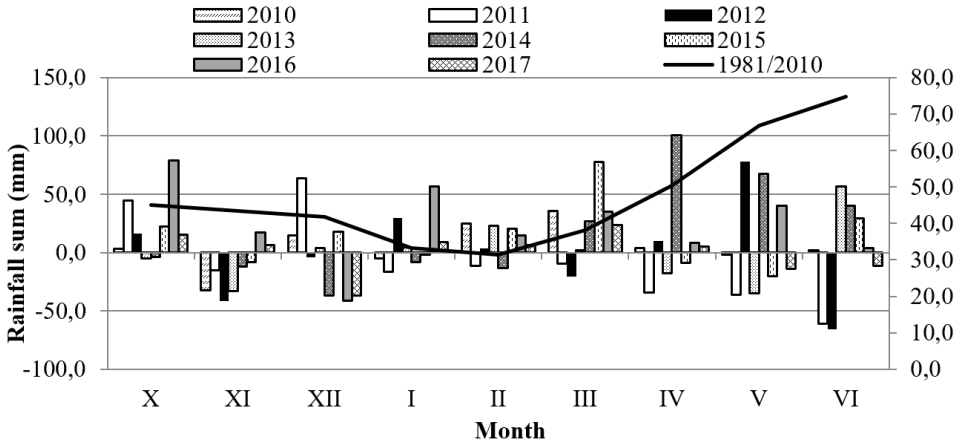


Figure 3. Deviation of monthly rainfall sum from climatic norm for the period 1981-2010 and climatic norm (1981-2010) in Sofia

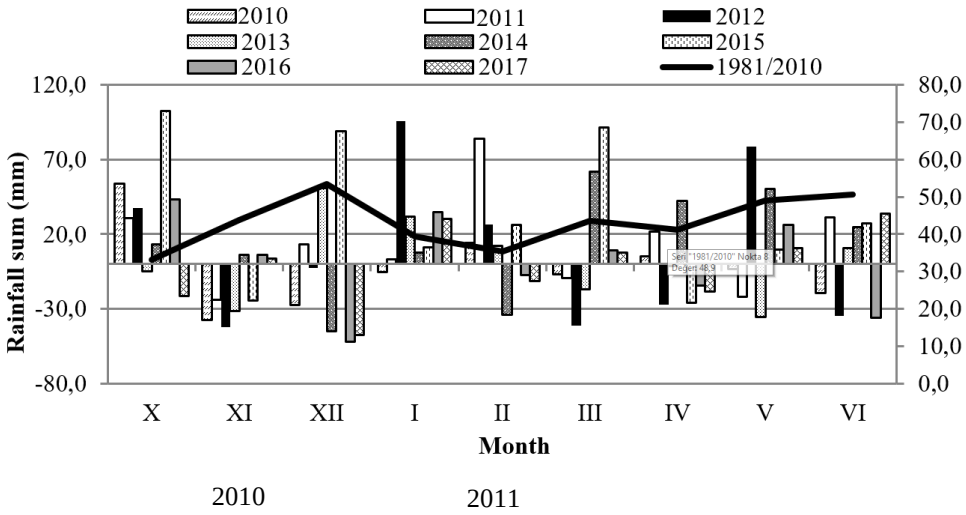


Figure 4. Deviation of monthly rainfall sum from climatic norm for the period 1981-2010 and climatic norm (1981-2010) in Chirpan

Results

Fungal spectrum

The results of this investigation carried out in the period from 2010 to 2017 showed that population of foliar fungal pathogens of durum wheat was heterogeneous and included 13 species (Table 1).

During the preliminary study performed in 2010 and 2011 only 46 diseased leaf samples were collected. Almost a half of specimens were from Chirpan and Sofia. The low disease level was observed in commercial fields due to regular fungicide treatments. *Pyrenophora tritici-repentis* (*Ptr*) was the most prevalent fungus. It was found in Chirpan and Sofia and all commercial fields. *Blumeria graminis* f. sp. *tritici* (*Pm*) and *Puccinia recondita* f. sp. *tritici* (*LR*) were present at most locations. *Parastagonospora nodorum* (*Pan*), *Zymoseptoria tritici* (*Ztr*), *Cochliobolus sativus* (*Cs*), *Puccinia striiformis* f. sp. *striiformis* (*YR*) and *Puccinia graminis* f. sp. *tritici* (*SR*) were not found at all. *Parastagonospora avenae* f. sp. *triticea* (*Pat*) was recorded only in Sofia. Besides it some unusual symptoms very similar to septoria leaf spot were also observed. On the leaves oval spots appeared with a lighter center, where fungal pycnidia developed in the concentric circles. Only the asexual morph was found. The fungus was determined as *Phaeophleospora* sp. (*Pps*) on the basis of morphological features. *Alternaria* sp. (*Alt*) was isolated from leaf samples collected in Chirpan and 5 of the commercial fields. A large number small light brown spots were observed in Chirpan and several commercial fields mainly on some foreign varieties of durum wheat as Levante and Karur (Italian) and Biensur (French). The lesions were oblong-oval with rounded edges, unclear contours and chlorotic halo. Some of them merged, giving relief to the diseased leaf surface. Sporulation was observed on the spots in humid conditions. The pathogen was identified as *Cladosporium herbarum* (*Ch*). A leaf disease of wheat caused by this fungus has not been reported so far in Bulgaria. *Monographella nivalis* (*Mn*) was found only in one commercial field near to Shumen town.

Altogether 1169 durum wheat leaves were examined in the period 2012-2017, including 738 and 431 from Sofia and Chirpan, respectively. The sum of the leaf samples bearing each of the studied pathogen was higher than the number of examined leaves because often the spots on one leaflet were caused by two and more different pathogens (Fig. 5).

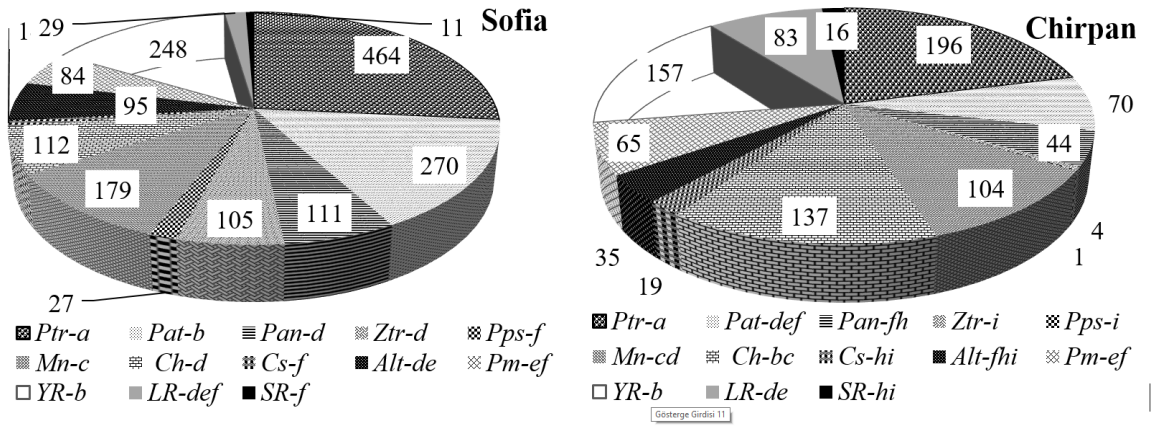
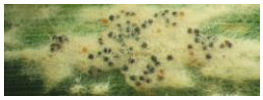
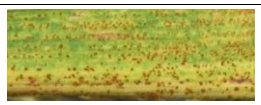


Figure 5. Occurrence of fungal pathogens in Sofia and Chirpan; the same letters after pathogens abbreviations mean no significant difference in their occurrence according to the Tukey-Kramer multiple range test (P≤0.05)

Along with asexual morph *Ptr, Pat, Mn* and *Ch* abundantly produced sexual one under climatic conditions of Bulgaria. Of the leaf spotting fungi, the causal agent of tan spot (*Ptr*) was the most prevalent in both prospected areas. The fungi belonging to Septoria leaf blotch complex (*Pat*, *Pan* and *Ztr*) and newly found species *Pps* were more frequently isolated from leaf samples taken in Sofia location. *Mn* and *Ch* also had contribution to the leaf spotting in both surveyed locations. *Cs* occurred only occasionally. Of the three rust fungi, *YR* was the predominant one.

Table 1. Fungal pathogens found during the investigation, the abbreviations and the characteristic symptoms of the respective diseases

Fungal pathogens	**Abbreviations**	**Symptoms**
Pyrenophora tritici-repentis (Died.) Drechs.	*Ptr*	
Parastagonospora avenae (A.B. Frank) Quaedvlieg, Verkley & Crous f. sp. *triticea* T. Johnson	*Pat*	
Parastagonospora nodorum (Berk.) Quaedvlieg, Verkley & Crous	*Pan*	
Zymoseptoria tritici (Desm.) Quaedvlieg & Crous	*Ztr*	
Phaeophleospora sp.	*Pps*	
Cladosporium herbarum (Pers. : Fr.) Link	*Ch*	
Monographella nivalis (Schaffnit) E. Müll.	*Mn*	
Cochliobolus sativus (S. Ito & Kurib.) Drechsler ex Dastur	*Cs*	
Alternaria sp.	*Alt*	
Blumeria graminis (DC) Speer f. sp. *tritici* Marchal	*Pm*	
Puccinia striiformis Westend. f. sp. *striiformis* Erikss.	*YR*	
Puccinia recondita Rob. ex Desm. f. sp. *tritici* Erikss. & Henn.	*LR*	
Puccinia graminis Pers. f. sp. *tritici* Erickss. & Henn.	*SR*	

The occurrence and relative prevalence of the fungal pathogens varied depending on the year of study. Agrometeorological conditions in some years were extremely favourable for the development of certain diseases. In Sofia, a total of 738 diseased leaves were collected in six consecutive years from 2012 to 2017. All 13 species surveyed were identified in 2017, but the most significant was the presence of *Ptr*, *Pan* and *Pat* (Fig. 6). In 2016 all pathogens occurred except *SR* with prevalence of *YR*, *Ptr* and

Pat. There was also a diversity of species in 2014 (10 species) and 2015 (8 species), with *Mn* dominating, followed by *Pat*, *Ptr* and *YR* in the first year and *Ptr* and *Ztr* in the second one. Two of the species (*Ptr* and *Pat*) appeared in all years studied, but with some prevalence of *Ptr*. *Pan*, *Ztr*, *Ch*, *Alt* and *YR* were recorded in five years.

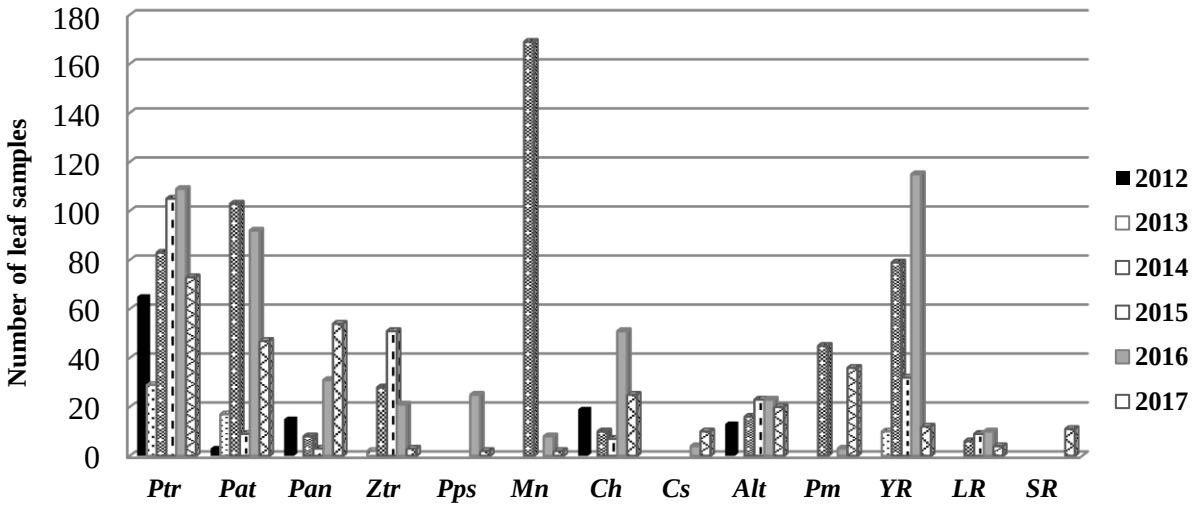


Figure 6. Occurrence of fungal pathogens on the leaves of durum wheat in Sofia location depending on the year of study

In Chirpan, a total of 431 diseased leaves were collected in the period 2012-2017 with the exception of 2015. The data on the occurrence of leaf pathogens depending on the year of examination are presented in Fig. 7. In 2013 and 2014 nine of the studied pathogens were present in the population but in the first one prevalent species were *Ptr*, *Ch* and *LR* and in the second one, *YR* and *Mn*. In 2017 eight pathogens were recorded with prevalence of *Ch*, *Pan*, and *Pat*. Interestingly, *Ptr*, one of the most common pathogen of durum wheat was not found in 2014 and 2017. Six species were established in 2016 of which *YR*, *Ptr* and *LR* were dominating. Two of the species (*Mn* and *Ch*) appeared in all years studied, but with different frequency. *Pm* and *YR* occurred in 4 years. *Ptr* was recorded in three of the years, but in a significant number of leaf samples. Unusually strong was the manifestation of *Pan* in 2017, as in previous years this fungus was isolated from the leaf of durum wheat in five samples only in 2014.

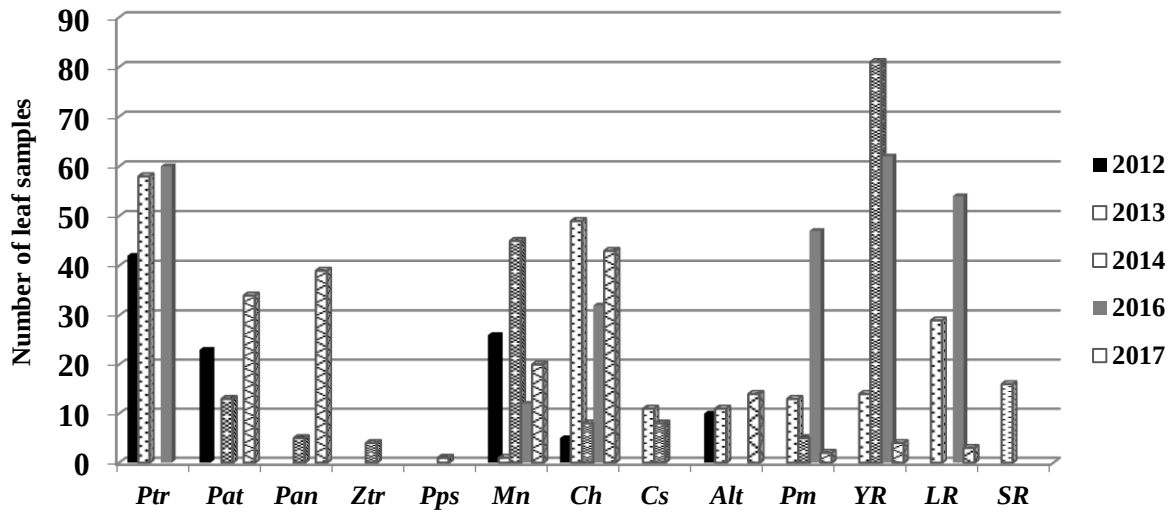


Figure 7. Occurrence of fungal pathogens on the leaves of durum wheat in Chirpan location depending on the year of study

The 12 varieties studied differed in their susceptibility to fungal diseases. The data for Sofia location are presented in Fig. 8. The appearance of Ptr was recorded for all varieties, but was more significant for Meridiano, Zvezditsa, Deyana and Auradur and less important - for Pescadou, Saragolla and GK Bétadur. Pat was the second most important species for the Sofia region. It appeared mostly on Zvezditsa, Progress and Auradur, and significantly less on Victoria. The strongest manifestation of Pan was on Zvezditsa, and of Mn and Ch – on Meridiano. Ztr was found in all varieties except GK Bétadur, but more noticeable in Zvezditsa and Vazhod.

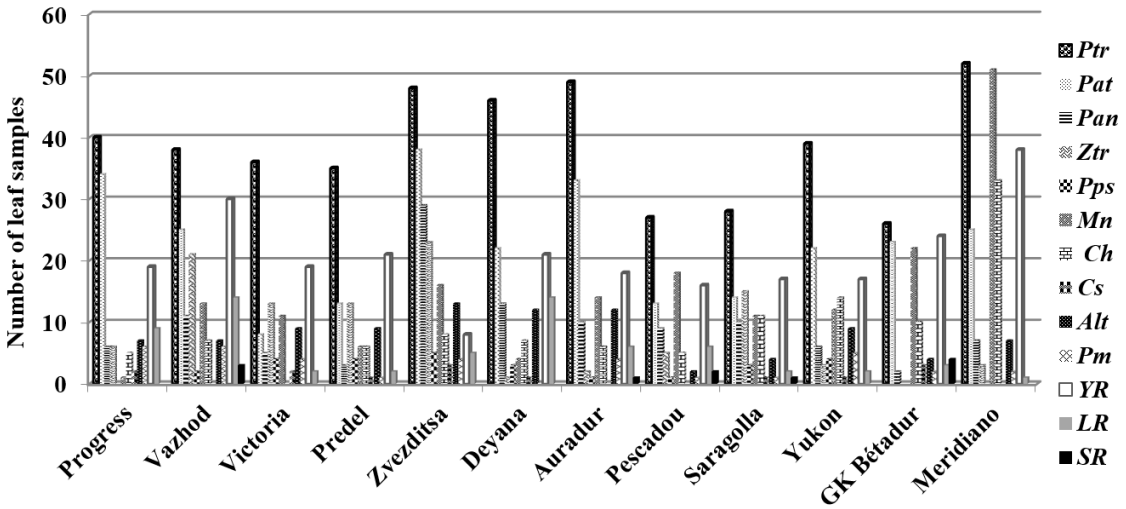


Figure 8. Occurrence of fungal pathogens on the leaves of durum wheat in Sofia location depending on the variety

In Fig. 9 are presented data on the appearance of foliar pathogens in Chirpan depending on the variety. *Ptr*, *Ch* and *Mn* were found in all varieties, but with a certain predominance of the first species on Zvezditsa and Auradur, of the second one on Auradur and Progress, and of the third one on GK

Bétadur and Yukon. *YR* occurred on all varieties with some prevalence on GK Bétadur and Yukon. *Pat* and *Pan* were isolated from all varieties except Meridiano.

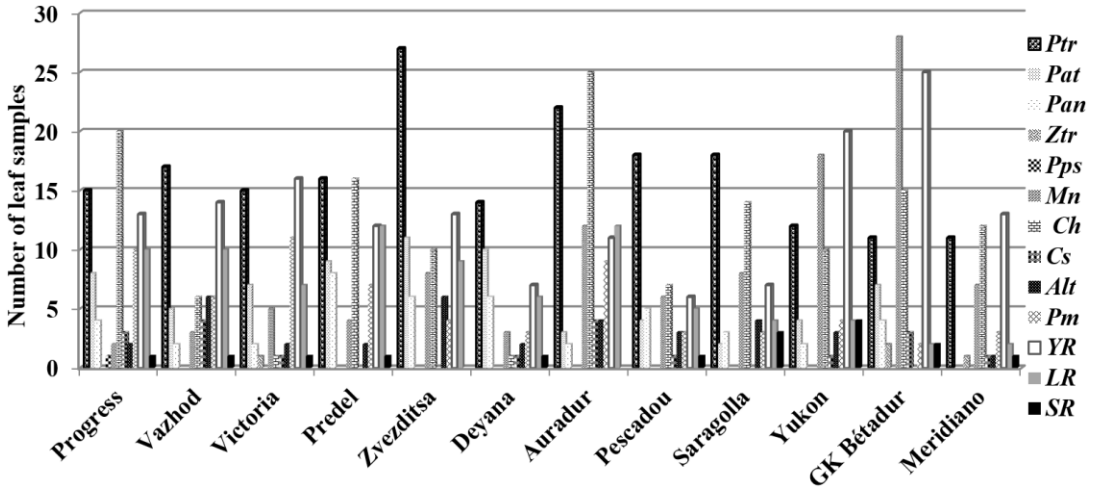


Figure 9. Occurrence of fungal pathogens on the leaves of durum wheat in Chirpan location depending on the variety

Discussion

During preliminary observations in 2010 and 2011 only a few pathogens were recorded. *Pan*, *Ztr*, *Cs*, *YR* and *SR* were not found at all. The long-term investigation performed for six (Sofia) and five (Chirpan) successive growing seasons enriched the fungal spectrum. The most prevalent on durum wheat were several fungal species (*Ptr*, *Pat*, *Mn* and *Ch*) that along with asexual morph produced abundantly sexual one under climatic conditions of Bulgaria. Both morphs of *Ch* have not been reported in Bulgaria so far as participants in the leaf spotting complex of wheat (*T. aestivum* and *T.durum*) but this fungus is a common pathogen of *T. aestivum* in Argentina (Perelló et al., 2003; Perelló, 2010). The ascomycetous fungus *Mn* primarily known as one of the causes of snow mold disease was observed in both locations, but most widely in the spring of 2012, 2014 and 2017 in Chirpan and 2014 in Sofia, providing a large amount of inoculum. In June when the leaf samples were collected the asexual and sexual morphs were commonly found together on the upper leaf level, including flag leaf.

Unusual symptoms similar to Septoria leaf spotting were noticed on durum leaves in both locations but more often in Sofia. During the studied period the asexual morph only was found. Morphologically, the pathogen was identified as *Phaeophleospora* sp. So far, the causal agent of wheat disease of this taxonomic group has not been reported in Bulgaria and the world. The fungi belonging to Septoria leaf blotch complex (*Pat*, *Pan* and *Ztr*) and newly found species *Phaeophleospora* sp. were more frequently isolated from leaf samples taken in Sofia location, with the most favorable weather conditions in 2014, 2016 and 2017 due to higher precipitations. Among them *Pat* was the prevalent species in both locations. Unusually strong performance of *Pan* was observed in Sofia in 2016 and 2017 and in Chirpan in 2017.

Of the three rust fungi, *YR* was the predominant one. The first record of yellow rust was made in 2013. It was the most common disease in both localities in 2014 and 2016 due to warmer winters, which favored the survival of YR. The race analysis performed in GRRC revealed the appearance and distribution of a new race (Warrior) in Bulgaria.

Except the weather other factors like cultivar characteristics, inoculum level, soil type, and predecessor played a role in changing spectrum of phytopathogenic fungi. In common the level of fungal diseases in Sofia region was higher compared to Chirpan. The studied varieties differed in their susceptibility to fungal diseases.

The results obtained for the fungal composition of the foliar diseases of durum wheat could be a good theoretical basis for further phytopathological and genetic studies on resistance to relevant pathogens.

Acknowledgemens

The authors greatly appreciate the race analysis of yellow rust held by Prof. Mogens Støvring Hovmøller and research group in Global Rust Reference Center, Denmark. Financial support by the Bulgarian National Science Fund (project DNTS Slovakia 01/5 is gratefully acknowledged.

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