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MONITORING OF BARLEY NET BLOTCH (*Pyrenophora teres* Drechsler) IN BULGARIA

Katya VASILEVA[1], Silviya VASILEVA[2], Milena KOSTOVA[2], Todorka SREBCHEVA[2]

[1]Maritsa Vegetable Crops Research Institute, Agricultural Academy,
32 Brezovsko Shosse Street, 4000, Plovdiv, Bulgaria
[2]Agricultural University - Plovdiv, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: silviya.valentinova@gmail.com

Abstract

Worldwide, net blotch caused by Pyrenophora teres Drechsler is a major foliar disease of barley (Hordeum vulgare L.) causing economic losses by reducing the yield and grain quality. Two forms P. teres f. maculata and P. teres f. teres have been identified as similar morphologically, however, different at the genetic and pathophysiological levels. The aim of the current study is to monitor the distribution of barley net blotch in Bulgaria. The total surveyed area during the investigation is 5212 dka. The lowest prevalence of the disease is in the regions of Imrenchevo and Chirpan, respectively 14.35% and 15.2%. The manifestation of net blotch is strongest in the region of Kamburovo - 82.2%. Based on the studied samples from different regions the species D. teres predominates in Bulgaria, while D. maculata is much rarer. The conidia of D. teres are light brown, cylindrical, with 3 to 8 partitions and dimensions - 69.5-181 x 15.28-24.1µm. The conidia of D. maculata are oblong-cylindrical with 3 to 5 partitions and with dimensions - 65-143 x 10.2-19.3 µm.

Key words: *Barley breeding, Hordeum vulgare, net blotch, pathogen resistance, Pyrenophora teres.*

INTRODUCTION

Worldwide, net blotch caused by *Pyrenophora teres* Drechsler [anamorph *Drechslera teres* (Sacc.) Shoem] is a major foliar disease of barley (*Hordeum vulgare* L.) causing economic losses by reducing the yield and the grain quality. Two forms *P. teres* f. *maculata* and *P. teres* f. *teres* have been identified as similar morphologically, however, different at the genetic and pathophysiological levels (Campbell et al., 1999; Liu et al., 2011; Akhavan et al., 2016).

P. teres f. *teres* forms dark-brown and longitudinal necrotic lesions, which can turn into chlorotic (Lightfoot & Able, 2010), while *P. teres* f. *maculata* is responsible for dark brown circular or elliptical spots with chlorosis on the surrounding leaf tissues (Gupta and Loughman, 2001; Jayasena et al., 2004). *Pyrenophora teres* f. *teres* (net form of net blotch (NFNB) and *Pyrenophora teres* f. *maculata* (spot form of net blotch (SFNB) are morphological very similar, while the disease symptoms are different (Akhavan et al., 2016). The pathogen of NFNB can be easily identified based on symptomatology, while SFNB presents symptoms without a net-spot (Marshall et al., 2015), which closely resemble those caused by *C. sativus* (Rehman et al., 2020).

Nowadays there are no studies on the prevalence of the disease in Bulgaria. The first data are given by Nakova (2009), according to reticular spots are little known as a disease in Bulgaria. The disease first appeared on a mass scale in the area of the town of Yambol in 2005 - on 5000 ha of crops, on a 0.2 to 0.5 ha patch. in the tillering phase.

The pathogenic factor and the quantity of primary inoculum from infected residues depend on several factors. Firstly, the environmental conditions and more specifically, long periods of wet increase the primary inoculum levels (Mclean et al., 2009). Secondly, the disease levels vary greatly depending on the cultural practice applied. Crop rotation, avoiding barley monoculture and eliminating or reducing primary inoculum in the field are means preventing the pathogen's development (Liu et al., 2011).

Geschele (1928) has first demonstrated the resistance to *P. teres* f. *teres* to be

quantitatively inherited (Clare et al., 2020). The genetic control of resistance to *P. teres* in barley has been first conducted in United States in 1955 (Afanasenko et al., 2007). The first gene *Pt1* conferring the barley resistance to *P. teres* is found by Schaller (1955). Later, two additional loci, designated *Pt2* and *Pt3* were identified by Mode & Schaller (1958). Initially, the genetics conferring resistance to *P. teres* f. *maculata* contained three major designated loci and therefore has been considered less complex to compare the *P. teres* f. *teres* – barley interaction (Clare et al., 2020).
The aim of the current study is to monitor the distribution of barley net blotch in Bulgaria.

MATERIALS AND METHODS

Spread of *Pyrenophora teres* in Bulgaria

A number of barley production areas in Bulgaria were evaluated for plants two leaf spot fungi reactions. The surveyed barley crops were located in the South Central, South Eastern and Northern parts of the country, typical areas for breeding the culture. The distribution was determined by examining the crops on randomly selected plants. The type of lesions occurring on plants was an indicator of susceptibility or resistance of cultivars to net blotch and spot-type net blotch in the field.
Cultivars susceptible to *P. teres* showed typical net-blotch lesions, having dark-brown striations extending both longitudinally and transversely within the lesions. In the case of spot-type net blotch, lesions varied from dark-brown spots to solid stripes, spreading longitudinally between leaf veins.
Periodic observations and macroscopic analyses were performed in order to detect the forms of the pathogen during the critical stages of barley to disease. The Tekauz scale for NFNB (net type) and SFNB (spot form) was used to determine the degree of infestation of the two forms of net blotch. The grades of 1-3 denote specimens resistant to SFNB, and 5-9 for susceptible in a different range. Respectively, values from 1 to 5 are conventionally considered as resistant forms to NFNB and 5 to 10 as sensitive (Figure 1).
The percentage of diseased plants compared to healthy ones was reported. The calculations were performed according to the formula of Chumakov (1974):

$$P = a/A*100,$$

Where: P - distribution, %
a - diseased plants
A - total number of reported plants
Observations of barley phenotype reactions to net blotch and fungus symptomatic manifestations were carried out under the field conditions, on different varieties of naturally infected plants. Field surveys described symptoms such as changes in plant habitus; change in colour, shape and size of lesions: chlorosis or yellowing, necrotic spots. Leaf samples, with symptoms of disease, were taken for isolation.

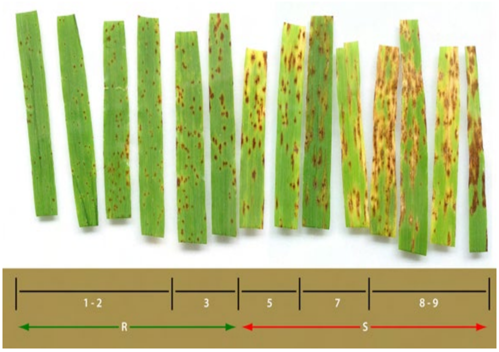

Figure 1. Tekauz's scale for determining the degree of attack by *Pyrenophora teres*

The presence of pathogens was proven macroscopically, microscopically on the basis of isolates on nutrient media by the accepted technique of biological analysis. Isolations were performed by naturally infected plant parts on Water agar (WA) and Potato Dextrose Agar (PDA).
Small pieces of infected tissues are cut from the border zone between the diseased and healthy part and then washed with running tap water. Isolates were prepared on PDA. From the pure cultures prepared, sporulation is examined, and pathogenicity tests are carried out by inoculation of healthy barley plants.
The obtained isolates pathogenicity was proven by inoculating barley plants in controlled conditions. Each variant was in three repetitions. The plants were grown in pots, on a sterile substrate. The plants were pulverized with the prepared spore suspension. The variants were placed in a humid chamber, periodically sprayed with water to maintain high humidity. Daily symptoms monitoring,

description and comparison with the control variants sprayed with sterile water was conducted. Re-isolation and microscopic analysis were performed as well. Plants were placed in a growth chamber at 25°C, RH 70% and periodically sprayed with water to maintain high humidity. If symptoms
appear, reisolation and microscopic analysis are carried out.
From the isolates, with proven pathogenicity, monospore cultures were obtained for morphological studies. Identification of the phytopathogen that cause the disease is performed macroscopically based on symptoms characteristics, and microscopically by morphological characters of the spores (Tafradjiiski et al., 1973). Morphological identification is based on the type of colonies (color, shape, type of mycelium), type of shape and size of spores.
The software products used during the study were “MS Excel Analysis Tool Pak Add-Ins” 2019 (https://support.office.com) and “R-4.0.3” in combination with “RStudio-0.98” and package installed “Agricolae 1,2-2” (Mendiburu, 2015).

RESULTS AND DISCUSSIONS

Net blotch has become a major foliar disease of barley not only in Bulgaria, but in many countries of the world (Backes et al., 2021). Based on the pathogen life cycle, three sources can form the primary inoculum of which infected seeds, crop debris, and straw residue. It is known that net blotch propagates efficiently on wild plants and, there is a huge reservoir of the pathogen in the wild host populations, which could occasionally or continuously serve as a source of initial inoculum to epidemics in the field (Ronen et.al., 2019). Therefore, the first step to control net blotch is the deletion of the primary inoculum of *P. teres* by sowing healthy seeds (Jalli, 2011).
Different areas are observed to determine the prevalence of barley disease *Drechslera teres* and *Drechslera maculata* in the country. The analysis of the data shows that the attack by regions varies widely. The results of the 2021 survey on the distribution of *Drechslera teres* and *Drechslera maculata* on barley in different regions of the country are shown in the Table 1 and Figure 2.
The total surveyed area in Bulgaria for the period of investigation was 5212 dka. On the surveyed areas was observed 40.54% spread of the disease. The variation was very pronounced between the different regions of the country.

Table 1. Distribution of *Drechslera teres* and *Drechslera maculata* on barley in some parts of the country in 2021

№	Region	Area (dka)	Diseased plants (%)
1	Aleksandrovo	150	37.45
2	Levka	22	48.25
3	Elhovo	140	22.3
4	Okop	210	19.5
5	Aheloy	60	36.2
6	Stozher	80	18.95
7	Karnobat	750	38.9
8	Chirpan	500	15.2
9	Imrenchevo	200	14.35
10	Milanovo	200	33.03
11	Lilyak 2	250	43.45
12	Izvorovo 1	350	52.50
13	Izvorovo 2	350	28.95
14	Kamburovo	150	82.2
15	Lilyak 2	300	55.64
16	Padarino	200	68.49
17	Zimnitsa	550	51.06
18	Dragoevo	450	50.00
19	Pleven Region	150	37.86
20	Vratza region	150	56.48

The standard deviation is 18.08%. The analysis of the reported data (Table 1) showed that the attack of the pathogen by regions varies widely.

Figure 2. *Pyrenophora teres* distribution of barley crops on the territory of Bulgaria

The lowest prevalence of the disease is in the regions of Imrenchevo (North Eastern

Bulgaria) and Chirpan (South Central part of the county), respectively 14.35% and 15.2%.
The manifestation of net form and spot form of net blotch was strongest in the region of Kamburovo (North Eastern) - 82.2%. Based on the studied samples from different regions, it was found that the species *Drechslera teres* (*Pyrenophora teres* f. *teres* - *Ptt*) predominates in the country while *Drechslera maculata* (*Pyrenophora teres* f. *maculata* - *Ptm*) is much rarer.
Gray fluffy mycelium with radial growth is formed on the PDA. Conidia are light brown, cylindrical, with 3 to 8 partitions and dimensions - 68.5-178 x 13.5-22.5µm (Figure 3).

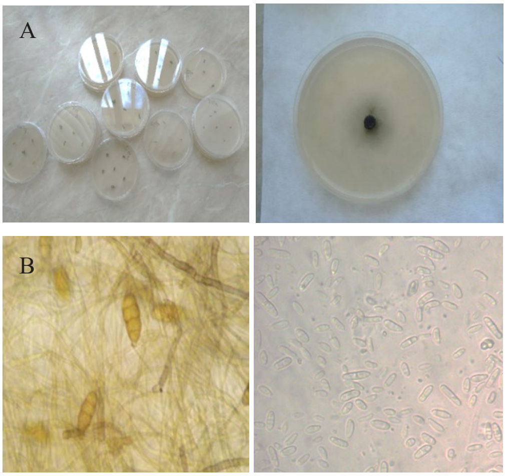

Figure 3. Conidia of *Pyrenophora teres* Drechsler [anamorph *Drechslera teres* (Sacc.) Shoem] on a petri dish (A) and from microscopic examination (B)

Similar results are also confirmed by Lammari et.al. 2019, in which Ptt is the dominating type of net blotch in Algeria and was prevalent in almost all provinces surveyed, while Ptm was found less frequently.
The differentiation of these forms has been reported from other authors in Sweden (Jonsson et al., 1997), France (Arabi et al., 2003), Western Australia (Gupta & Longhman, 2001), South Africa (Lowu et al., 1996), and Western Canada (Akhavan et al., 2016).
Surveys of foliar diseases in barley (Hordeum vulgare) crop throughout Victoria Australia indicated spot form of net blotch caused by *Pyrenophora teres* f. *maculata* had the highest incidence, being present in more than 90% of crops surveyed (McLean et. al., 2010).

In 2021, 20 isolates of *Drechslera teres* and *Drechslera maculata*, which are pathogenic for the culture, were obtained from different regions and different cultivars. All inoculations were prepared of naturally infected leaves, on AA and PDA.
The spores of *Drechslera teres* form in groups of 2 to 3, and emerge through the stomata. The conidia of *D. teres* are light brown, cylindrical, with 3 to 8 partitions and dimensions - 69.5-181 x 15.28-24.1µm (Figure 3). The conidia of *D. maculata* are oblong-cylindrical with 3 to 5 partitions and with dimensions - 65-143 x 10.2-19.3µm.
Light brown spots in the form of a network were observed. The mass manifestation of the disease is in the growth stage of tillering at a temperature of 18 to 20ºC and precipitation. Depending on the variety, small, dark brown rounded to elliptical spots were formed on the leaf blade, surrounded by a lighter crown, or the characteristic brown-red spots, interspersed with transverse and longitudinal lines, which give them a reticulated appearance.
The leaves wither from the top to the base and die in a short time. At high humidity, the sporulation of the fungus was formed on the spots, in the form of a dark spore-bearing deposit (Figure 4).

Figure 4. Pathogenicity of isolates evaluated under controlled condition with high humidity

Several methods for disease assessment are applied for monitoring of *P. teres* infection. The evaluation of the disease in the laboratory and under controlled condition, where *P. teres* isolates is sprayed with a sprayer on the leaves at different stages of growth after sowing in order to follow the disease development. After pathogen inoculation, the barley plants are placed in a hood to increase the humidity level, allowing the pathogen to improve its development (Backes et al., 2021). The disease can also be followed on detached leaves, a rapid technique of assessment under controlled conditions (El-Mor et al., 2017).

A reliable method used for monitoring and scoring of resistance to barley net blotch, so called ‘‘summer hill trial’’ was developed in which winter barley is sown at the beginning of August at optimum conditions for *P. teres* infection and in order to other diverse pathogen infections with *Rhynchosporium commune*, *Puccinia hordei* or *Blumeria graminis* f. sp. *hordei* to be avoided (König et.al. 2013).

During this study, we were able to confirm field observations in controlled conditions on barley plants using the fungal isolates. The seedling symptoms rate under artificial inoculation of commercial barley varieties formed the varietal structure in the country was 100%.

CONCLUSIONS

Pathogenicity tests and morphological studies as well as analyses indicated that the described symptoms were caused by the forms of *Pyrenophora (Drechslera) teres*. Recognized as the causal agent of net blotch, *Pyrenophora teres* is responsible for major losses of barley crop yield. The consequences of this leaf disease are due to the impact of the infection on the photosynthetic performance of barley leaves.

The susceptibility of barley cultivars currently being grown in Bulgaria, variable distribution and *P. teres* barley pathosystem, indicates that incorporating resistance and identifying new resistant germplasm should remain a high priority.

It’s still challenged the better understanding of local isolate pathogenicity, epidemiology, and host–pathogen interactions, which are needed to breed more resistant cultivars

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