

RESEARCH PAPERS

Pathotypes of *Pyrenophora teres* on barley in Turkey

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Summary. Net blotch foliar diseases of barley are important in Turkey, lowering grain yields and quality. There are two forms, the spot form (caused by *Pyrenophora teres* f. *maculata* (*Ptm*)) and the net form (caused by *P. teres* f. *teres* (*Ptt*)). To determine the pathotypes of *Ptt* and *Ptm* in Turkey, surveys were carried out during 2012, 2013 and 2015. *Pyrenophora teres* samples were collected from 34 provinces of Turkey. From these samples, 258 *Ptm* and 167 *Ptt* single conidium isolates were obtained. Pathotypes of 50 *P. teres* f. *maculata* and 40 *P. teres* f. *teres* isolates were assessed by inoculating onto a differential set of 25 barley genotypes. Twenty six *Ptm* pathotypes and 24 *Ptt* pathotypes were identified, and significant pathogenic variation was found among the isolates. Barley breeding programmes in Turkey should consider the pathotypes identified for incorporation of net blotch resistance. Continuous virulence monitoring for the *P. teres* population should be carried out to inform resistance breeding priorities.

Key words: Barley, *Drechslera teres* f. *maculata*, *Drechslera teres* f. *teres*.

Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop in Turkey, being the second most planted cereal after wheat (Tuik, 2016). Barley is cultivated in 2.598 million ha, producing 6.31 million tonnes of grain, at an average of 2,450 kg ha⁻¹ (Tuik, 2016). Net blotch diseases, caused by the fungus *Pyrenophora teres* (anamorph: *Drechslera teres*) are important foliar diseases of barley, which limit barley production by reducing grain yield and quality (Mathre, 1982; McLean *et al.*, 2009; Liu *et al.*, 2011). There are two main net blotch diseases: the spot form caused by *Pyrenophora teres* f. *maculata* (*Ptm*), and the net form caused by *Pyrenophora teres* f. *teres* (*Ptt*) (Smedegard-Petersen, 1971). Symptoms of the spot form consist of necrotic spots surrounded by chlorosis (McLean *et al.*, 2009; Liu *et al.*, 2011). The net form symptoms consist of thin, dark brown, longitudinal streaks on leaves which merge to create irregular streaks on leaves (Liu *et al.*, 2011).

These diseases can cause significant grain yield and quality losses (Mathre, 1982; Aktaş, 1997; Karakaya *et al.*, 2014). Yield losses can reach up to 100% in severely affected fields where very susceptible cultivars are grown, but generally losses are between 10–40% (Mathre, 1982).

Planting resistant barley cultivars is an effective way of controlling the net blotch diseases. However, both *Ptm* and *Ptt* show pathogenic variation and have the potential to overcome host resistance. Pathogenic variation needs to be considered in plant breeding programmes (Tekauz, 1990; Liu *et al.*, 2011; Çelik Oğuz and Karakaya, 2015; Akhavan *et al.*, 2017). The pathogenic variation in *P. teres* has been known since 1949 (Pon, 1949). Khan and Boyd (1969) used differential barley lines to determine the physiological races of *D. teres*. Later studies reported pathogenic variation in both forms of *P. teres* populations from different parts of the world. These studies utilized different lines for variation studies, and large variation among the *P. teres* populations were reported (Khan and Tekauz, 1982; Harrabi and Kamel, 1990; Steffenson and Webster, 1992b; Sato and Takeda, 1993; Jonsson *et al.*, 1997; Platz *et al.*, 2000; Arabi *et al.*, 2003; Cromey and Parkes, 2003; Wu *et al.*, 2003;

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Tuohy *et al.*, 2006; Afanasenko *et al.*, 2009; Lehmen-siek *et al.*, 2010; McLean *et al.*, 2011; Boungab *et al.*, 2012; McLean *et al.*, 2014; Leišová-Svobodová *et al.*, 2014; Akhavan *et al.*, 2017).

In the present study, 50 *Ptm* and 40 *Ptt* isolates were tested on 25 differential barley test cultivars and genotypes under greenhouse conditions, to determine the pathotypes of these fungi in Turkey.

Materials and methods

Survey and collection of *Pyrenophora teres* isolates

Two hundred and seventy nine barley fields in 2012, 105 in 2013 and 71 in 2015, were surveyed in 34 provinces of Turkey. Fields were sampled at distances of approx. 30 km, within different regions of the country (Aktaş, 2001). Leaves with spot form and net form symptoms were sampled in each field.

Single conidium isolates, isolate selection and verification of isolates

Leaves containing net or spot form symptoms were cut into small pieces, 2–5 cm in length, and surface sterilized by placing in 1% sodium hypochloride solution for 1 min. Leaf pieces were then placed onto Petri dishes containing sterile moistened filter paper and incubated for 3 d for conidium production. Single conidia were individually placed onto water agar. Hyphal tips from germinating conidia were transferred to potato dextrose agar (PDA) to develop cultures. Two hundred and fifty eight *Ptm* and 167 *Ptt* single conidium isolates were obtained from different regions of Turkey. From these, 90 isolates (50 *Ptm* and 40 *Ptt* isolates) were selected. These isolates were obtained from 23 provinces of Turkey, including: Edirne, Denizli, Afyon, Eskişehir, Ankara, Konya, Çankırı, Kırıkkale, Aksaray, Kırşehir, Mersin, Kayseri, Kilis, Kahramanmaraş, Sivas, Gaziantep, Diyarbakır, Şanlıurfa, Mardin, Şırnak, Siirt, Batman and Adıyaman. Isolates were chosen based on their geographic separation, size of barley cultivation area in respective provinces, and isolate morphological characteristics (growth rate, colour, growing habit) in agar cultures. The identities of the isolates were verified for their net and spot form status by inoculating cultures onto local barley cv. Bülbül 89, which is susceptible to net and spot forms of the pathogen (Karakaya *et al.*, 2014; Usta *et al.*, 2014; Yazıcı *et al.*, 2015).

Differential host set

The differential set outlined by Wu *et al.* (2003) was used for pathotype determination of both forms of *P. teres*. This set consisted of 25 barley genotypes. Twenty two of these were used by Steffenson and Webster (1992b) in an earlier study.

Inoculation, incubation and disease assessments

Five to ten seeds of each differential set genotype were planted in 7 cm diam. plastic pots containing a mixture of top soil, sand and organic matter (60:20:20, v:v:v). Plants were maintained in greenhouse conditions before and after inoculation. Three replicates of each genotype were sown to pots. They were arranged in a randomized fashion. Inoculum of each single conidium isolate was obtained from a 10-d-old culture grown on PDA, by scraping the culture with a paintbrush and washing through cheesecloth with water. Inoculum density, consisting of mycelium pieces, was adjusted to $1.5\text{--}2.0 \times 10^5$ mycelium parts per mL. One drop of Tween 20 was added to each 100 mL of inoculum suspension (Aktaş, 1995). Seedlings were inoculated at the two to three leaf stage (Z12-13; Zadoks *et al.*, 1974). Mycelium suspensions were sprayed individually onto sets of seedlings, and the inoculated plants were kept at high humidity in closed transparent lid boxes for 76 h in a greenhouse. The temperature of the greenhouse was $18\text{--}23\pm 1^\circ\text{C}$ with a 14h/10h light/dark regime. After this period, the box lids were opened for 48 h under the same conditions. After 7 d, the seedlings were assessed for disease severity using the net and spot form scales described by Tekauz (1985).

Pathotype determination

The differential set of barley genotypes were numbered from 1 to 25, as follows: 1 = Tifang, 2 = Canadian L. Shore, 3 = Atlas, 4 = Rojo, 5 = Coast, 6 = Manchurian, 7 = Ming, 8 = CI 9819, 9 = Algerian, 10 = Kombar, 11 = CI 11458, 12 = CI 5791, 13 = Harbin, 14 = CI 7584, 15 = Prato, 16 = Manchuria, 17 = CI 5822, 18 = CI 4922, 19 = Hazera, 20 = Cape, 21 = Beecher, 22 = Rika, 23 = NDB 112, 24 = FR 926-17, and 25 = Hector.

The genotypes that scored as 1, 2, 3, 4, 5 according to Tekauz (1985) scale were evaluated as resistant (R); whereas those that scored as 6, 7, 8, 9, 10 were evaluated as susceptible (S).

The pathotype terminology described by Steffenson and Webster (1992b) and Wu *et al.* (2003) was used. Each number in a pathotype assay corresponds to the numbered virulence type of the isolate, which is virulent (severity scale values 6–10). The isolates that were not virulent (scale values 1–5) to all the differential set genotypes were identified as Pathotype 0 (Wu *et al.*, 2003).

Results

Pathotypes

From 50 *Ptm* isolates and 40 *Ptt* isolates, 26 *Ptm* and 24 *Ptt* pathotypes were determined on the 25 differential barley genotypes (Tables 1 and 2).

The most common pathotype among the *Ptm* isolates was Pathotype 6-18, represented by 12 isolates (Figure 1). The other common pathotypes were Pathotype 0 and Pathotype 18, which consisted of seven isolates, corresponding to 14% of total isolates each. The most complex pathotype, Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25 (isolate Gps 263) was virulent to all 25 of the tested differential barley genotypes.

The most common pathotype among *Ptt* isolates was Pathotype 0 which was represented by seven isolates (Figure 1). The most complex pathotype, Pathotype 3-4-6-7-9-10-11-12-14-15-16-17-18-20-21-22-25 (isolate Gps 18) was virulent to 17 of the tested differential barley genotypes.

Differential set

Differential genotype CI 4922 was susceptible to 34 *Ptm* isolates (68% of total *Ptm* isolates). Cultivar Manchurian gave susceptible reactions to 25 *Ptm* isolates (50% of *Ptm* isolates) and cv. Kombar was susceptible to 21 *Ptm* isolates (42% of *Ptm* isolates). No genotype was resistant to all *Ptm* isolates, although genotype NDB 112 was resistant to 48 *Ptm* isolates and susceptible to only two *Ptm* isolates. Cultivar Prato and genotype FR 926-17 were resistant to 45 *Ptm* isolates and susceptible to five of these isolates.

Cultivar Kombar was susceptible to 29 *Ptt* isolates (73% of the *Ptt* isolates). Genotype CI 4922 was susceptible to 26 *Ptt* isolates (65% of *Ptt* isolates) and cv. Manchurian was susceptible to 22 *Ptt* isolates (55% of *Ptt* isolates). Cultivar Tifang and genotypes NDB 112 and FR 926-17 were resistant to all of the

Ptt isolates. Also, cvs. Ming, Harbin, Manchuria and CI 5791 genotype were susceptible to only one *Ptt* isolate, and resistant to 39 of these isolates.

Discussion

This is the first detailed study of virulence of *Pyr-enophora teres* f. *teres* and *P. teres* f. *maculata* populations in Turkey. The populations were pathogenically diverse, with 26 pathotypes identified for *Ptm*, and 24 identified for *Ptt*.

In previous studies, researchers identified numerous pathotype/isolate ratios of *Ptt*. Pathotype/isolate ratios varied between 0.14 and 1 (Tekauz, 1990; Steffenson and Webster, 1992b; Jonsson *et al.*, 1997; Douiyssi *et al.*, 1998; Cromey and Parkes, 2003; Wu *et al.*, 2003; Bouajila *et al.*, 2011; Fowler and Platz, 2011; Boungab *et al.*, 2012; Liu *et al.*, 2012; Akhavan *et al.*, 2016). In our study, the pathotype/isolate ratio for *Ptt* was 0.6. This pathogenic variation was less than reported by Douiyssi *et al.* (1998), Wu *et al.* (2003) and Liu *et al.* (2012), but greater than reported for the other studies mentioned above.

In previous *Ptm* pathotype determination studies, Karki and Sharp (1986) recognized six groups, and Gupta *et al.* (2012) recognized seven groups. In other studies, pathotype/isolate ratios varied between 0.47 and 0.55 (Tekauz, 1990; Wu *et al.*, 2003; McLean *et al.*, 2014; Akhavan *et al.*, 2016). In our study, the pathotype/isolate ratio of *Ptm* was 0.52. This variation was less than that of McLean *et al.* (2014), but greater than in the other studies mentioned above.

Serenius *et al.* (2007) reported that pathogenic and genetic structures of *Ptm* populations could be different in every continent. According to McLean *et al.* (2011), there were different reactions of different host genotypes to isolates from Australia and Canada, and even for pathogen isolates from the same continent. Other studies showed that the resistance to both net and spot pathogen forms can change when alternating barley cultivars are planted (Khan, 1982; Gupta and Loughman, 2001; Cromey and Parkes, 2003).

Although there are several studies for the spot form of this pathogen, studies on the net form have been more common, since the net form is more prevalent globally (Louw *et al.*, 1996; McLean *et al.*, 2009; Liu and Friesen, 2010). In our survey, net and spot forms of *P. teres* were found, but the spot form was more common (Karakaya *et al.*, 2014). Several

Table 1. Twenty six pathotypes of *Pyrenophora teres* f. *maculata* determined in Turkey.

Isolate No.	Location	Susceptible genotypes No./ Pathotype No.
13-181	K.Maraş Pazarcık	Pathotype 0
13-157 <i>H. spontaneum</i>	Diyarbakır Central District	
Gps 49	Kayseri Tomarza	
13-177	Adıyaman Gölbaşı	
Gps 68	Kırşehir Central District	
13-167 <i>H. spontaneum</i>	Diyarbakır Central District	
Gps 265	Ankara Ş.Koçhisar	
Gps 116	Konya Bozkır	Pathotype 18
Gps 3	Ankara Elmadağ	
Gps 81	Çankırı İlgaz	
13-116	Niğde Ulukışla	
Gps 79	Çankırı Central District	
Gps 270	Konya Ereğli	
Gps 129	Konya Cihanbeyli	
Gps 90	Ankara Haymana	Pathotype 6-18
13-114	Aksaray Central District	
Gps 125	Konya Karatay	
Gps 101	Konya Akşehir	
Gps 122	Konya Çumra	
Gps 272	Mersin Central District	
13-194	Kayseri İncesu	

(Continued)

Table 1. (Continued).

Isolate No.	Location	Susceptible genotypes No./ Pathotype No.
13-149 <i>H. spontaneum</i>	Mardin Midyat	
Gps 50	Kayseri Tomarza	
Gps 187	Eskişehir Beylikova	
Gps 158	Eskişehir Odunpazarı	
Gps 227	Eskişehir Sivrihisar	
Gps 70	Kırşehir Kaman	Pathotype 5-21
Gps 8	Kırıkkale Delice	Pathotype 5-18
13-139 <i>H. spontaneum</i>	Mardin Central District	Pathotype 3-10
Gps 119	Konya Güneysınır	Pathotype 6-10-18
Gps 162	Eskişehir Alpu	
Gps 177	Ankara Nallıhan	Pathotype 6-10-18-22
Uhk 74	Gaziantep Kargamış	Pathotype 5-12-14-21
Gps 99	Konya Yunak	Pathotype 6-10-11-13-18
13-142	Mardin Ömerli	Pathotype 1-3-5-9-10-11-22
Gps 43	Kayseri Bünyan	Pathotype 10-11-13-15-18-22-25
Edirne	Edirne	Pathotype 2-5-7-9-10-13-18-21
Gps 27	Sivas Şarkışla	Pathotype 2-4-5-6-10-11-12-13-14-18
13-168	Diyarbakır Central District	Pathotype 5-8-10-11-12-14-19-20-21-22
Gps 155	Afyon Emirdağ	Pathotype 2-4-5-6-10-11-12-13-14-17-18-19-25
13-136	Mardin Nusaybin	Pathotype 3-5-6-7-9-10-11-14-19-20-21-22
Gps 19	Sivas Central District	Pathotype 4-5-6-8-10-11-13-16-17-18-22-24-25

(Continued)

Table 1. (Continued).

Isolate No.	Location	Susceptible genotypes No./ Pathotype No.
13-163	Diyarbakır Central District	Pathotype 1-2-3-5-8-9-10-11-13-14-20-21-22
13-122	Şanlıurfa Central District	Pathotype 1-2-3-5-8-9-10-11-14-15-16-19-20-21-22-25
Gps 276 <i>Hordeum bulbosum</i>	Kilis Central District	Pathotype 1-2-3-4-5-7-8-9-10-11-12-13-14-18-19-20-22-24-25
13-127 <i>Hordeum spontaneum</i>	Şanlıurfa Ceylanpınar	Pathotype 1-2-3-4-5-6-7-8-9-10-11-13-14-15-16-17-20-21-22-25
Gps 76	Ankara Kalecik	Pathotype 1-2-3-4-5-6-8-9-10-11-12-14-15-16-17-18-19-20-21-22-23-25
13-167	Diyarbakır Central District	Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-16-18-19-20-21-22-24
13-179	Kahramanmaraş Pazarcık	Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-17-18-19-20-21-22-24-25
Gps 263	Ankara Bala	Pathotype 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21-22-23-24-25

researchers have used different differential host sets for this type of study. Some of these sets included the same barley cultivars for spot and net form of the disease. In these studies, several common differential lines were used (Karki and Sharp, 1986; Tekauz, 1990; Gupta and Loughmann, 2001; Wu *et al.*, 2003), and comparisons of global virulence variations have been made (Afanasenko *et al.*, 2009). In the present study, we employed the differential set used by Wu *et al.* (2003), and this was useful for revealing the pathotypes of both forms of *P. teres*.

Cultivar Kombar was used as a susceptible control cultivar in previous studies (Steffenson and Webster, 1992a; Steffenson and Webster, 1992b; Cromey and Parkes, 2003). This cultivar was susceptible to more than half of the isolates tested in the present study.

Cromey and Parkes (2003) found the barley genotype CI 4922 to be resistant to all isolates tested, whereas Steffenson and Webster (1992a) and Wu *et al.* (2003) reported this genotype to be susceptible to some pathotypes. In our study, genotype CI 4922 was susceptible to 68% of *Ptm* and 65% of *Ptt* isolates tested.

Cultivar Tifang and genotypes NDB 112 and FR 926-17 were resistant to all *Ptt* isolates tested in the

present study. Genotype CI 5791 was resistant to all except one isolate, namely isolate Gps 18. A similar result was reported by Akhavan *et al.* (2016), where genotype CI 5791 was resistant to all but one isolate tested. Furthermore, Afanasenko *et al.* (2009) and Fowler *et al.* (2014) emphasised that genotype CI 5791 was highly resistant. Cultivar Tifang was a resistant control cultivar in the Cromey and Parkes (2003) study, and exhibited a resistant reaction. Also, Steffenson and Webster (1992b) reported that cv. Tifang was resistant to all Californian *P. teres* pathotypes.

In the case of our *Ptm* isolates, host genotype NDB 112 was susceptible to two isolates (4%) and resistant to 48 isolates. Genotype FR 926-17 was susceptible to five isolates and resistant to 45 isolates (10%), whereas cv. Tifang and genotype CI 5791 were susceptible to nine (18%) isolates and resistant to 41 isolates. Tekauz and Mills (1974) indicated that genotype CI 5791 was less resistant to the spot form of barley net blotch disease.

Wu *et al.* (2003) reported that cvs. Rojo and Coast, and genotypes CI 9819, CI 5791, CI 7584, CI 5822, NDB 112, FR 926-77 were resistant to all *Ptt* and *Ptm* isolates they tested. In our study, from 50 *Ptm* isolates; two isolates (4%) were virulent on genotype

Table 2. Twenty four pathotypes of *Pyrenophora teres* f. *teres* determined in Turkey

Isolate No.	Location	Susceptible genotypes No./ Pathotype No.
Gps 134	Eskişehir Tepebaşı	Pathotype 0
15-61	Gaziantep Şahinbey	
15-41	Siirt Central District	
13-134	Mardin Kızıltepe	
Denizli	Denizli	
Gps 271	Mersin Central District	
13-172	Diyarbakır Central District	
13-174	Adıyaman Central District	Pathotype 22
13-111	Ankara Ş.Koçhisar	Pathotype 18
13-123	Şanlıurfa Central District	Pathotype 2-10
15-66	Kilis Central District	Pathotype 6-22-25
Gps 167	Eskişehir Seyitgazi	Pathotype 6-10-18
15-48	Batman Central District	
Gps 205	Eskişehir Sivrihisar	
Gps 33	Sivas Gemerek	
15-60	Gaziantep Şahinbey	
Gps 145	Eskişehir İnönü	
Gps 198	Eskişehir Mahmudiye	Pathotype 2-6-10-18
Gps 213	Eskişehir Çifteler	Pathotype 6-10-18-25
Gps 53	Kayseri Kocasinan	
Gps 243	Eskişehir Sivrihisar	Pathotype 6-10-18-20

(Continued)

Table 2. (Continued).

Isolate No.	Location	Susceptible genotypes No./ Pathotype No.
15-62 <i>Hordeum spontaneum</i>	Kilis Central District	Pathotype 6-10-18-22-25
15-39 <i>Hordeum spontaneum</i>	Siirt Tillo	
15-13	Ankara Yenimahalle	Pathotype 3-6-10-18-20-25
15-65	Kilis Central District	
Uhk 67	Şanlıurfa Birecik	
Gps 110	Konya Meram	Pathotype 2-6-9-10-18-25
15-37	Şırnak Cizre	Pathotype 3-6-10-18-22-25
15-26	Şanlıurfa Ceylanpınar	Pathotype 2-6-9-10-18-25
Gps 201	Eskişehir Mahmudiye	Pathotype 3-5-6-9-10-17-18-25
Gps 48	Kayseri Tomarza	Pathotype 2-3-5-6-9-18-21-25
13-126	Şanlıurfa Central District	Pathotype 2-3-8-10-17-18-19-20-21
15-32	Mardin Central District	Pathotype 2-3-6-9-10-15-18-19-20-21
13-151	Mardin Midyat	Pathotype 2-3-4-8-9-10-14-18-19-20
13-175	Adıyaman Besni	Pathotype 3-4-8-9-10-11-15-17-20-21
13-130	Şanlıurfa Ceylanpınar	Pathotype 2-3-8-9-10-11-14-15-18-19-20-21
Uhk 77	Kilis Central District	Pathotype 2-3-4-5-9-10-11-13-14-17-18-19-20-21-22-25
Gps 18	Sivas Yıldızeli	Pathotype 3-4-6-7-9-10-11-12-14-15-16-17-18-20-21-22-25

NDB 112, five isolates (10%) on genotype FR 926-17, six isolates (12%) on genotype CI 5822, nine (18%) on genotype CI 5791 and cv. Rojo, ten (20%) on genotype CI 9819, 13 (26%) on genotype CI 7584, and 18 isolates (36%) were virulent on cv. Coast. From 40 *Ptt* isolates; five (12.5%) were virulent on genotype CI 5822, four (10%) on cv. Rojo and genotypes

CI 9819 and CI 7584, three (7.5%) on cv. Coast, and one isolate was virulent on genotype CI 5791. The genotypes NDB 112 and FR 926-77 were found resistant to all of the *Ptt* isolates. Tekauz and Mills (1974) reported that resistant hybrid lines CI 5791 and BT 201 were resistant to the net form of *P. teres*, but less resistant to the spot form in production areas. In an-

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