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Mating type idiomorphs of *Pyrenophora teres* in Turkey

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Abstract

Pyrenophora teres f. *maculata* (*Ptm*) and *Pyrenophora teres* f. *teres* (*Ptt*) causes spot form and net form of net blotch diseases of barley, respectively. Although both forms of *P. teres* are morphologically similar, their symptoms and genetic background differ. In this study, 175 single spore (109 *Ptm* and 66 *Ptt*) isolates obtained from different regions of Turkey were evaluated for their mating type distribution and prevalence. Fungal isolates of both forms were verified using species-specific polymerase chain reaction (PCR) primers. For mating type determination studies, duplex PCR was performed using MAT-specific single nucleotide polymorphism primers. Sixty and 49 of 109 *Ptm* isolates were found as *MAT1-1* and *MAT1-2* types, respectively and 43 and 23 of 66 *Ptt* isolates were found as *MAT1-1* and *MAT1-2* types, respectively. These results show the possibility of sexual reproduction among the *Ptm* isolates in Turkey and *Ptt* population of Central Anatolia, Turkey. However, the overall pattern of *Ptt* isolates did not support the sexual reproduction hypothesis in Turkey. Sexual reproduction in the life cycle of *P. teres* is important since it could lead to genetic and pathogenic variation. As a result of new sexual combinations more virulent pathotypes of *P. teres* may occur.

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

Introduction

Pyrenophora teres Drechsler (anamorph: *Drechslera teres* (Sacc.) Shoem.) causing net blotch has two morphologically similar forms, *P. teres* f. *maculata* (spot form of net blotch) and *P. teres* f. *teres* (net form of net blotch) (Smedegård-Petersen, 1971; Liu et al., 2011). Symptoms of the spot form of net blotch consist of small necrotic spots surrounded by chlorosis (McLean et al., 2009; Liu et al., 2011). Symptoms of the net form of net blotch consist of thin, dark brown, longitudinal streaks on leaves. Later, these streaks merge and create irregular streaks on leaves (Liu et al., 2011). Net blotch of barley plants is an important foliar disease in the world. The pathogens cause significant reductions in yield and quality of barley worldwide (Murray, Brennan, 2010). General losses are between 10–40% although this percentage is much higher in the fields where susceptible barley cultivars are grown (Mathre, 1982). Resistant cultivars, crop rotation and fungicide application are some ways to manage net blotch (Turkington et al., 2011).

A single mating type (*MAT*) locus controls two mating types (*MAT1-1* and *MAT1-2*) in heterothallic ascomycetes (Kronstad, Staben, 1997; Debuchy, Turgeon, 2006). These two alleles are called idiomorphs (Rau et al., 2005). For pseudothecia formation two fungal strains with different idiomorphs are necessary (Kronstad, Staben, 1997; Rau et al., 2005; Sommerhalder et al., 2006).

Pyrenophora teres is a self-sterile and heterothallic ascomycete fungus and needs to have two mating types for sexual reproduction (Kronstad, Staben, 1997). Both forms of net blotch produce sexual pseudothecia and asexual conidia. In the sexual cycle of the fungus ascospores are produced in pseudothecia which are over-wintering in infected plant debris. In the asexual cycle, airborne conidia spread and cause multi-cyclic disease (Liu et al., 2011).

Sexual recombination occurs naturally within the net form and spot form isolates, separately in each form of the fungus. In order for sexual reproduction to occur, which is a major source for primary infections, the two mating types have to be present at statistically equal frequencies (Rau et al., 2005; Bogacki et al., 2010). The two mating types found in unequal frequencies are associated with high probability of asexual reproduction through conidia (Sommerhalder et al., 2006; Bogacki et al., 2010). Hybridization between the spot form and the net form, on the other hand, is either very rare or non-existent in nature; however, it is possible under laboratory conditions (Campbell et al., 2002).

Sexual reproduction in *P. teres* populations does indeed occur (Rau et al., 2005; Bogacki et al., 2010; Fiscor et al., 2014; Akhavan et al., 2015). However, Lehmensiek et al. (2010) reported that in some *P. teres* populations,

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reproduction was mainly asexual. Knowledge pertaining to multiplication through sexual reproduction in net blotch pathogens is crucial to understanding their evolutionary potential (Sommerhalder et al., 2006). The presence of both mating types and sexual recombination could lead to novel genotypes of the fungus. The novel strains of the fungus may as well be more virulent than the present strains. Both *Ptm* and *Ptt* isolates show pathogenic variation and have the potential to overcome resistances. Numerous studies have reported pathogenic variation in both forms of net blotch populations from various parts of the world (Lehmensiek et al., 2010; Boungab et al., 2012; McLean et al., 2014; Akhavan et al., 2016; Çelik Oğuz, Karakaya, 2017).

Barley is the second most important cereal crop produced in Turkey after wheat. It is mostly cultivated in the Central Anatolia followed by the Southeast Anatolia. Southeast Anatolia is one of the major gene centers of barley as a part of Fertile Crescent where cereals were firstly cultivated in the world. Surveys revealed that both forms of *P. teres* are present in Turkey; however, the spot form appears to be more common (Karakaya et al., 2014). In addition, several researchers have reported the pathogenic variation of *P. teres* to be high (Yazıcı et al., 2015; Çelik Oğuz, Karakaya, 2017). Nonetheless, there has been no study to date on the presence and prevalence of mating types of both forms of *P. teres* in Turkey.

In this study, MAT-specific single nucleotide polymorphism (SNP) primers were used to determine the presence and frequency of mating types of *Pyrenophora teres* f. *maculata* and *P. teres* f. *teres* for the first time in Turkey. We also tested the hypothesis of multiplication of net blotch populations by sexual reproduction. A preliminary result of this study has been published (Çelik Oğuz et al., 2017).

Materials and methods

Collection of Pyrenophora teres isolates and obtaining single spore isolates. During the 2012–2013 and 2015–2016 growing seasons 270 diseased leaf samples were collected from barley fields at about every 30 kilometres in 28 different provinces (Manisa, Uşak, Denizli, Afyon, Edirne, Eskişehir, Konya, Aksaray, Ankara, Niğde, Kayseri, Nevşehir, Kırşehir, Çankırı, Kırıkkale, Yozgat, Sivas, Şırnak, Batman, Siirt, Mardin, Diyarbakır, Şanlıurfa, Adıyaman, Kilis, Gaziantep, Kahramanmaraş and Mersin) of Turkey (Fig. 1). From each field, 1–5 samples of diseased leaves were taken. Leaves exhibiting net and spot type symptoms were cut into small pieces and subjected to 1 minute of surface sterilization with 1% sodium hypochlorite. For obtaining single spores standard blotter technique for spore production was used (ISTA, 1996). Single spores were taken under a stereomicroscope and were placed onto potato dextrose agar (PDA). The majority of barley growing areas in Turkey are located in Central and Southeast Anatolia regions. Ninety percent of the isolates belong to these two regions. During the surveys *P. teres* f. *maculata* (*Ptm*) were more commonly observed. Among these 270 leaf samples, 175 samples (105 *Ptm* and 70 *Ptt*) were selected and single spore isolations were made. The two biotypes of pathogen were visually separated based on disease symptoms (Tekauz, 1985). For selection of these samples large barley planting areas of these two regions were considered. For each form of net blotch, isolates were selected from the fields representing the same field or different fields. During the 2015–2016 growing season, samples were taken from the Central and Southeast Anatolia regions of Turkey. In this growing season no samples were taken from the Marmara, Aegean and Mediterranean regions.

Figure 1. Collection locations of barley leaves infected with *Pyrenophora teres* f. *maculata* and *P. teres* f. *teres* in Turkey

Genomic DNA extraction from fungal cultures. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method modified by Allen et al. (2006). Briefly, cell walls of fungal mycelia were broken down by grinding tissue with MagNA lyser (Roche, Germany). The CTAB extraction buffer: 0.1 M Tris, pH 8.0, 1.4 M NaCl, 0.02 M ethylenediaminetetraacetic acid (EDTA), 5 g of CTAB, 0.5–1% (v/v) of β-mercaptoethanol (βME), was added and samples were then incubated at 65°C for 30 min. After centrifugation, the supernatant was transferred into new 2 mL tubes including phenol:chloroform:isoamyl alcohol (25:24:1). After new centrifugation step, aqueous phase was put into new tube and cold isopropanol was added. Precipitated DNA was solved in Tris-EDTA (TE) buffer (0.01 M Tris, pH 8.0 and treated with DNAse free RNAse-A at 37°C for 30 min). After a series of ethanol precipitation steps, DNA was cleaned up and allowed to

air dry. Finally, the DNA was dissolved in 200 μl of pure water and adjusted to a final concentration of 20 ng μL^{-1} in TE and stored at −20°C.

Species-specific and mating type form-specific duplex polymerase chain reaction (PCR) assays. PtGPD1 (control) primer pair (586 bp) was used for the species-specific PCR analysis (Lu et al., 2010) (Table 1). PCR reaction mixture was prepared as 25-μL final volume containing 20 ng of fungal genomic DNA, 1 μM each primer, 1× MyTaq reaction buffer (15 mM $MgCI_2$ and 5mM dNTPs), 0.125 units Taq DNA Polymerase (Bioline, USA). Cycling conditions were 95°C for 3 min, 35 cycles of 15 s at 95°C, 15 s at 55°C and 10 s at 72°C with a final extension of 72°C for 30 s. *Pyrenophora tritici-repentis* isolate was used as a negative control.

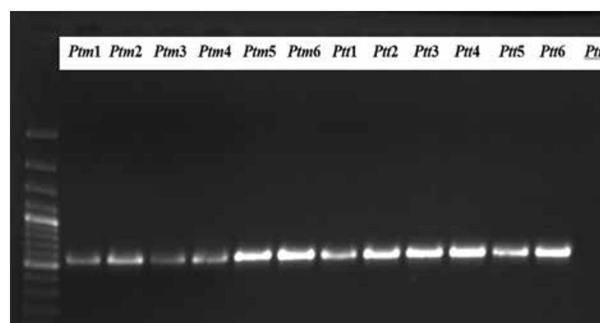
Diseased leaf samples were identified as spot or net forms of net blotch according to symptoms on barley leaves in the field. Assumed net blotch isolates were tested with *P. teres* species-specific primers in order to separate from other pathogens such as *Cochliobolus sativus*. A mating type form-specific duplex PCR analysis was performed using a set of single nucleotide polymorphism (SNP) primers *Ptt MAT1-1*F/R, *Ptt MAT 1-2* F/R and *Ptm MAT1-1* F/R, *Ptm MAT1-2* F/R, described by Lu et al. (2010); PCR products: net form – *Ptt MAT1-1* (1.143 bp) and *Ptt MAT 1-2* (1.421 bp); spot form – *Ptm MAT1-1* (194 bp) and *Ptm MAT1-2* (939 bp) (Table 1). These primer pairs are also form-specific primers for *Ptm* and *Ptt*. Duplex PCR was performed separately for each form. PCR products mentioned in this study also differentiate between *Ptm* and *Ptt* isolates. PCR reaction mixture and cycling conditions were the same as described in the species-specific PCR assay with the exception of annealing temperature which was increased to 59°C.

Table 1. Single nucleotide polymorphism (SNP) primers used in this study (Lu et al., 2010)

Primer name	Primer sequence	Amplicon size
PtGPD1-F	CGTATCGTCTTCCGCAAC	586 bp
PtGPD1-R	TTGGAGAGCACCTCAATGT	
Ptt MAT1-1 F	ATGAGACGCTAGTTCAGAGTCT	1143 bp
Ptt MAT1-1 R	GATGCCCAGCCAAGGACAA	
Ptt MAT1-2 F	TACGTTGATGCAGCTTTCTCAAT	1421 bp
Ptt MAT1-2 R	AACACCGTCCAAAGCACCT	
Ptm MAT1-1 F	TGTTAGAGACCCCACCAGCGT	194 bp
Ptm MAT1-1 R	CAGCTTTCTTGGCCTTCTGAA	
Ptm MAT1-2 F	ACGCAAGGTACTCTGTACGCA	939 bp
Ptm MAT1-2 R	GACGTCGAGGGAGTCCATTT	

PCR products were loaded on 1% agarose gels containing ethidium bromide with a concentration of 0.1 μg ml^{-1} and electrophoresed in 0.5× Tris-borate-EDTA (TBE) for 1.5 h at 115 V. Gels were photographed under UV light Quantum ST4 (Montreal Biotech, Canada). The sizes of the fragments in a gel were compared with GeneRuler 100 bp DNA ladder Plus (MBI Fermentas, USA).

Statistical analysis. The χ^2 significance test was used to determine whether the rate of mating type observed for *P. teres* populations in Turkey was clearly distinguished from the null hypothesis of the 1:1 ratio of MAT1:MAT2. The formula in which χ^2 is calculated: $\chi^2 = \sum[(o - e)^2/e]$, where o is the observed value of the mating type, e – the expected value of the mating type (Sommerhalder et al., 2006).



Note. Ptm – Pyrenophora teres f. *maculata*, *Ptt – P. teres* f. *teres*, *Ptr – P. tritici-repentis*; last line is negative control.

Figure 2. Species-specific PtGPD1 (control) primer pair (586 bp) used in this study for confirmation of *Pyrenophora teres* isolates

Results and discussion

One hundred seventy-five single spore isolates were obtained from different regions of Turkey and they were confirmed to be *P. teres* using the species-specific PtGPD1 (control) primer pair (Fig. 2).

The presence of both *MAT1-1* and *MAT1-2* mating types in both forms of net blotch in Turkey was observed using a mating type form-specific duplex PCR assay. Also, form-specific SNP primers used in our current study separated two forms of net blotch (Fig. 3). The two biotypes of pathogen were visually separated based on disease symptoms. The two biotypes of the samples that were separated as net form of net blotch or spot form of net blotch were subjected to form-specific duplex PCR.

As a result of this study, 3 isolates (2.85%) previously identified as *Ptm* were determined as *Ptt* and 7 isolates (10%) previously identified as *Ptt* were determined as *Ptm*. Thus, 109 single spore isolates of *Ptm* from 24 different provinces and 66 single spore *Ptt* isolates from 18 different provinces were obtained. Of the 109 *Ptm* isolates, 76 were obtained from Central Anatolia, 23 from Southeast Anatolia, 6 from Mediterranean, 3 from Aegean and 1 from Marmara regions of Turkey. Of the 66 *Ptt* isolates, 44 were obtained from the Central Anatolia, 20 from the Southeast Anatolia, 1 from Marmara and 1 from the Aegean regions of Turkey (Table 2).

MAT1-1 and *MAT1-2* ratios of *Ptm* isolates during the 2012–2013 and 2015–2016 growing seasons

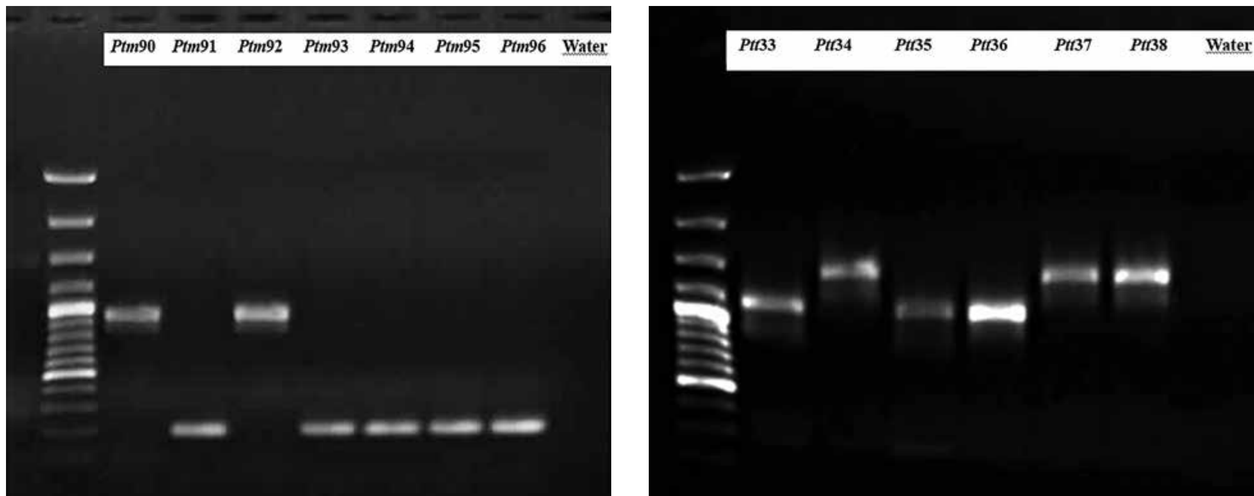


Figure 3. Multiplex PCR analysis of mating types of *Pyrenophora teres* f. *maculata* (*Ptm*) and *P. teres* f. *teres* (*Ptt*) in Turkey with single nucleotide polymorphism (SNP) primers *Ptm MAT1-1*, *Ptm MAT1-2* and *Ptt MAT1-1*, *Ptt MAT 1-2*

Table 2. One hundred and seventy-five *Pyrenophora teres* (109 *Ptm* and 66 *Ptt*) isolates used in this study and their respective regions

Growing seasons	Regions									
	Marmara		Aegean		Mediterranean		Southeast Anatolia		Central Anatolia	
	Ptm	*Ptt*	*Ptm*	*Ptt*	*Ptm*	*Ptt*	*Ptm*	*Ptt*	*Ptm*	*Ptt*
2012–2013	1	1	3	1	6	–	8	5	30	14
2015–2016	–	–	–	–	–	–	15	15	46	30
Total	1	1	3	1	6	–	23	20	76	44

in Mediterranean, Southeast and Central Anatolia regions supported sexual reproduction hypothesis. However, only *Ptt* samples obtained from Central Anatolia region supported this hypothesis. In some cases, both mating types (*MAT1-1* and *MAT1-2*) were found in samples taken from the same field (Tables 3–6).

Table 3. Mating type distribution of *Pyrenophora teres* f. *maculata* populations obtained from different regions of Turkey during 2012–2013 and 2015–2016 growing seasons

Growing seasons	Regions									
	Marmara		Aegean		Mediterranean		Southeast Anatolia		Central Anatolia	
	MAT1-1	*MAT1-2*	*MAT1-1*	*MAT1-2*	*MAT1-1*	*MAT1-2*	*MAT1-1*	*MAT1-2*	*MAT1-1*	*MAT1-2*
2012–2013	1	–	3	–	2	4	4	4	17	13
2015–2016	–	–	–	–	–	–	8	7	25	21
Total isolates	1	–	3	–	2	4	12	11	42	34

Table 4. Mating type distribution and frequencies of *Pyrenophora teres* f. *maculata* populations obtained from different regions of Turkey

Regions	*MAT1-1*	*MAT 1-2*	χ^2 (1:1) types (df = 1)	*P*
Southeast Anatolia	12	11	0.0435	0.8348
Marmara	1	–	–	–
Mediterranean	2	4	0.6667	0.4142
Aegean	3	–	–	–
Central Anatolia	42	34	0.8421	0.3588
Total	60	49	1.1101	0.2921

df – degrees of freedom

Of the 109 *Ptm* isolates, 60 (55.05%) were found to be *MAT1-1*, while 49 (44.95%) were found to be *MAT1-2*. Of the 66 *Ptt* isolates, 43 (65.15%) were found to be *MAT1-1*, while 23 (34.85%) were found to be *MAT1-2*. Among *Ptm* isolates, ratios of *MAT1-1* and *MAT1-2* were similar, whereas among *Ptt* isolates, the idiomorph *MAT1-1* was found to be more common.

The χ^2 test confirmed that all of *Ptm* isolates were consistent with the 1:1 null hypothesis. It was observed that the hypothesis was also confirmed for the

Table 5. Mating type distribution of *Pyrenophora teres* f. *teres* populations obtained from different regions of Turkey during 2012–2013 and 2015–2016 growing seasons

Growing seasons	Regions							
	Marmara		Aegean		Southeast Anatolia		Central Anatolia	
	MAT1-1	*MAT1-2*	*MAT1-1*	*MAT1-2*	*MAT1-1*	*MAT1-2*	*MAT1-1*	*MAT1-2*
2012–2013	–	1	1	–	4	1	9	5
2015–2016	–	–	–	–	11	4	18	12
Total isolates	–	1	1	–	15	5	27	17

Table 6. Mating type distribution and frequencies of *Pyrenophora teres* f. *teres* populations obtained from different regions of Turkey

Regions	*MAT1-1*	*MAT 1-2*	χ^2 (1:1) types (df = 1)	*P*
Southeast Anatolia	15	5	5.0000	0.0253*
Marmara	–	1	–	–
Aegean	1	–	–	–
Central Anatolia	27	17	2.2727	0.1317
Total	43	23	6.0606	0.0138*

df – degrees of freedom; * – significantly different mating-type frequencies that deviate from a 1:1 ratio (P = 0.05)

Central and Southeast Anatolia and Mediterranean regions. The finding that the isolates showed no deviation from the 1:1 ratio supports the hypothesis that the fungus *P. teres* f. *maculata* reproduces sexually in its life cycle in Turkey.

In the overall mating type patterns of *Ptt* isolates and in the isolates from the Southeast Anatolia regions, departures from the 1:1 ratio were observed. Isolates from the Central Anatolia region, on the other hand, showed no deviation from the 1:1 ratio. This finding suggests that in Turkey, fungus *P. teres* f. *teres* reproduction type (sexual or asexual) may depend on the region. While the results suggest that the fungus reproduces sexually in the Central Anatolia region, this may not be the case in the Southeast Anatolia region where the results support the hypothesis that the pathogen reproduces asexually. Limited sample size obtained from regions other than Central Anatolia region may be responsible for these results. Serenius et al. (2005) reported that 1:1 ratio was obtained when sample size increased.

In order to assess the sexual recombination potential of *P. teres*, PCR-based mating type studies have been shown to be beneficial by many researchers (Rau et al., 2005; Serenius et al., 2005; Beattie et al., 2007; Lu et al., 2010; Akhavan et al., 2015). Rau et al. (2007) emphasized that in future studies the two forms should be evaluated separately and that well-defined net form or spot form isolates should be used to screen the barley genotypes. SNP primers used in our study were able to distinguish the two forms. SNP primers previously used by Lu et al. (2010) were used in our study. They stated that these primers successfully distinguished the two forms and were more useful than the *PttMAT*- and *PtmMAT*-related SNPs used in previous studies.

Researchers from various regions of the world have reported different results regarding the mating frequency distributions in *P. teres* populations. Rau et al. (2005) reported that sexual reproduction was at significant levels in both forms of *P. teres* in Sardinia, while Bogacki et al. (2010) suggested that the asexual component should not be neglected although reproduction was mainly sexual in the *Ptt* and *Ptm* populations in South Australia. Fiscor et al. (2014) reported that in *Ptt* populations in Hungary, idiomorphs of both mating types observed to be similar in frequency and that this should increase the sexual reproduction potential. Akhavan et al. (2015) concluded that in Canada, the two mating type genes were observed in equal frequencies in both forms of net blotch populations and the leading primary inoculum source was via sexual reproduction. On the contrary, Lehmensiek et al. (2010) reported that asexual reproduction was the dominant means of reproduction in both the *Ptm* and *Ptt* populations obtained from South Africa and the whole of Australia.

Peever and Milgroom (1994) observed random sexual reproduction in Alberta, North Dakota and German populations, but not in the New York population. Jonsson et al. (2000) pointed out that while in the *P. teres* cultivar 'Svani' population asexual reproduction was observed, on the overall pattern, the Swedish population might have a sexual reproduction cycle. Serenius et al. (2007) observed only the *MAT2* idiomorph in Krasnodar (Russia), however, found the mating type ratio (*MAT1* and *MAT2*) to be 1:1 in several locations in Australia and Finland. Statkevičiūtė et al. (2010) found both mating types in Lithuania *Ptt* populations with a 1:1 ratio with the exception of Klaipėda population. These studies showed that the distribution and genetic differentiation of *P. teres* sexual reproduction were quite variable between different geographical regions. Also in our current study, the mating type ratio in the *Ptt* population showed variation from region to region. While the 1:1 hypothesis was shown to be true for the Central Anatolia region, in the remaining regions, it was not the case.

In a given region, the mating type ratio of the pathogen might fluctuate over the years. Robinson and Jalli (1996), Jalli and Robinson (2000) and Robinson and Mattila (2000) reported that the pathogen reproduced mainly asexually in Finland; however, Serenius et al.

(2005) reported that the mating type ratio in Finland did not deviate from 1:1 and sexual reproduction was common among the Finnish *P. teres* populations. Their study was the first to observe sexual reproduction in *P. teres* in Finland.

The only study on the biology of the spot form of net blotch in Turkey was performed by Karakaya et al. (2004). In this study, infected leaves collected from the Central Anatolia region were kept under and above ground. Pseudothecia of the pathogen were observed on the leaves left on the ground and buried, but no ascospores were detected. This finding supports the hypothesis that in the Central Anatolia region in Turkey, the *P. teres* f. *maculata* can reproduce sexually. There has been no study on the biology of the *Ptt* in Turkey. The presence and frequency of mating types in both forms have been reported with the current study for the first time in Turkey.

Mutation, population size and random genetic drift, gene and genotype flow, reproduction and mating system, selection imposed by major gene resistance and quantitative resistance affect pathogen evolution. Understanding the factors that affect pathogen evolution will help to develop control strategies against net blotch. When the pathogen population is small, the mutations are not as important as sexual reproduction. It has been reported that if the asexual spores of the pathogen fungus are able to travel long distances, a high risk for causing an epidemic through migration and gene flow is possible (Burdon, Silk, 1997; McDonald, Linde, 2002). However, in *P. teres*, the potential of conidial spread is thought to be limited. In fungi, reproduction is observed to be either sexual, asexual or mixed in places where both sexual and asexual reproduction is present. Pathogens with mixed reproduction systems (sexual + asexual) are believed to carry the most risk for evolution (McDonald, Linde, 2002). In Turkey, *P. teres* is believed to have a mixed reproduction system as shown in our study in which in addition to asexual reproduction, *P. teres f. maculata* also exhibited sexual reproduction in its life cycle all around Turkey (Karakaya et al., 2004, and this study), while *P. teres f. teres* exhibited sexual reproduction in the Central Anatolia region.

Genotype variation is high in pathogen populations that exhibit sexual reproduction. Many new combinations of alleles in sexual reproduction lead to novel genotypes and this is one of the most important risks for control of the pathogen because the novel genotypes can be more virulent than the previous ones and overwhelm the hosts' genetic durability. Methods that prevent development of sexual reproduction have been implicated as possible disease management strategies (McDonald, Linde, 2002; Beattie et al., 2007). In Turkey, 26 *Ptm* and 24 *Ptt* pathotypes have been discovered, suggesting that pathogenic variation was high (Çelik Oğuz, Karakaya, 2017).

Mating type surveys are the most practical method to assess the sexual and recombination potential in some species. In heterothallic species, populations that only have one mating idiomorph cannot mate randomly. However, if they do mate randomly, the mating ratio is expected to be 1:1. It is observed without exception that sexual populations have more genetic diversity than asexual populations in the same fungal species. Rejection of null hypothesis in mating type tests means that there is no random mating in the population. However, caution should be exercised about reaching conclusions related to sexual reproduction in the populations. Rejection of the random mating hypothesis means that the recombination can occur in a regular manner (Milgroom, 1996).

Conclusions

1. This is the first study dealing with mating types of both forms of *Pyrenophora teres* in Turkey.

2. *P. teres* f. *maculata* (*Ptm*) was found more common in Turkey. We obtained 109 (62.2%) *Ptm* and 66 (37.8%) *P. teres* f. *teres* (*Ptt*) isolates. The majority of the samples were taken from Central and Southeast Anatolia regions of Turkey where most barley production occurs.

3. Form-specific single nucleotide polymorphism (SNP) primers successfully separated two *P. teres* forms. As a result of form-specific duplex PCR study, 3 (2.85%) isolates previously identified as *Ptm* were determined as *Ptt*, and 7 (10%) isolates previously identified as *Ptt* were determined as *Ptm.*

4. Both mating types of spot and net forms of *P. teres* have been found in Turkey. It appears that *Ptm* population ($\chi 2$ (1:1) types = 1.1; $P = 0.29$) in Turkey and Central Anatolia population of *Ptt* ($\chi 2$ (1:1) types = 2.2; $P = 0.13$) have a mating type with similar frequencies and these support the sexual reproduction hypothesis. However, *Ptt* population in Turkey ($\chi 2$ (1:1) types = 6.06; $P = 0.01$) did not approximate to 1:1 ratio. Limited sample size obtained from regions other than Central Anatolia region may be responsible for these results.

5. Central Anatolia region is an important barley production area in Turkey. Sexual reproduction in *P. teres* appears to be significant in certain regions of Turkey since conidia of *P. teres* cannot travel long distances. As a result of sexual reproduction, fungus may produce more virulent pathotypes in the future. Using mating type surveys to monitor the pathogen and comparing isolates collected with long time intervals with each other will make follow-up of genetic variations possible and will help in cultivation of durable plants as well as adoption of effective control strategies.

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Pyrenophora teres dauginimosi tipo idiomorfos Turkijoje

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Santrauka

Pyrenophora teres f. *maculata* (*Ptm*) ir *Pyrenophora teres* f. *teres* (*Ptt*) sukelia dėmėtąją ir tinkliškąją miežių dryžligę. Nors abi *P. teres* formos yra morfologiškai panašios, jų simptomai ir genetinė kilmė skiriasi. Tyrimo metu vertintas 175 vienos sporos (109 *Ptm* ir 66 *Ptt*) izoliatų, gautų iš įvairių Turkijos regionų, dauginimosi tipas ir jų tarpusavio santykis. Abiejų formų grybų izoliatai tirti naudojant polimerazės grandininės reakcijos (PGR) rūšiai specifinius pradmenis. Dauginimosi tipas nustatytas taikant dvigubą PGR, dauginimuisi naudojant MAT specifinius pradmenis. Iš 109 *Ptm* izoliatų buvo nustatyta 60 MAT1-1 ir 49 MAT1-2 tipai, o iš 66 *Ptt* izoliatų – 43 MAT1-1 ir 23 MAT1-2 tipai. Tyrimo rezultatai rodo lytinio dauginimosi galimybę tarp *Ptm* izoliatų Turkijoje ir *Ptt* populiacijos Centrinėje Anatolijoje, Turkijoje. Tačiau bendra *Ptt* izoliatų struktūra nepatvirtino lytinio dauginimosi hipotezės Turkijoje. Lytinis dauginimasis *P. teres* gyvenimo cikle yra svarbus, nes jis gali lemti genetinę ir patogeninę variaciją. Dėl naujų lytinio dauginimosi kombinacijų gali atsirasti virulentiškesni *P. teres* patotipai.

Reikšminiai žodžiai:, *Drechslera teres* f. *maculata*, *Drechslera teres* f. *teres*, miežiai.