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Full Length Article

Genetic Diversity of Net Blotch Pathogens of Barley in Turkey

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Abstract

Pyrenophora teres f. *maculata* (*Ptm*) (spot form of net blotch) and *Pyrenophora teres* f. *teres* (*Ptt*) (net form of net blotch) are common leaf pathogens that cause net blotch disease of barley. From different regions of Turkey during the years 2012-2016 and 2017, 48 *Ptt* and 49 *Ptm* single spore isolates were obtained from different *Hordeum* species [*H. vulgare* (40 *Ptm*, 47 *Ptt*), *H. spontaneum* (7 *Ptm*, 1 *Ptt*) and *H. bulbosum* (2 *Ptm*)]. The genetic diversity of the isolates was accomplished using inter simple sequence repeat (ISSR) marker system. Using the species-specific and mating-type specific PCR primers, 48 *Ptt* and 49 *Ptm* isolates were identified as 30 *MAT 1-1*, 18 *MAT1-2* and 27 *MAT1-1*, 22 *MAT1-2* types, respectively. In order to determine genetic diversity of isolates, 10 ISSR primers were used and seven of these primers showed optimal band numbers and intensities. The most useful primers were UBC 825 and UBC 807 for *Ptt* population and UBC 825, UBC 807 and UBC 827 for *Ptm* population. The similarity rate of the *Ptm* isolates was found to be lower than that of *Ptt* isolates. There was no definite separation by year, mating type or geographical area. However, *Ptt* population was clustered more by location. The similarity rate of isolates obtained from wild barleys collected from Southeast Anatolia region of Turkey located in the Fertile Crescent region were low. Although some isolates from the same wild hosts and provinces showed low similarity, some isolates obtained from different wild hosts showed higher similarity to each other. This variation in isolates from wild barleys may contribute to the genetic diversity of the *P. teres* population. Genetic diversity of *Ptt* and *Ptm* populations in Turkey was assessed for the first time with this study. Investigation of pathogen biology and genetic structure will be useful for the development of disease control strategies.

Keywords: *Drechslera teres*; Genetic diversity; ISSR; Net blotch; *Pyrenophora teres*

Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated plants grown in Anatolia for thousands of years (Kün, 1996). It is the second most important cereal crop following wheat in Turkey (Tuik, 2017). Turkey is one of the important gene centers of barley. Southeast Anatolia region of Turkey, located in the Fertile Crescent region where barley, wheat, lentil, hard-seeded fruits and olive are cultivated for the first time, is an important barley growing area (Kün, 1996; Brown *et al*., 2009). In Turkey, wild barleys and barley landraces are also common (Karakaya *et al*., 2016; Çelik Oğuz *et al*., 2017; Ergün *et al*., 2017). Wild barleys and barley landraces are considered as 'gold mines' for different stress tolerance factors and they are new useful genetic variation sources (Ceccarelli and Grando, 2000; Çelik and Karakaya, 2017; Ergün *et al*., 2017). The ancestor of cultivated barley *Hordeum spontaneum* C. Koch is an important gene source and it grows naturally in Turkey (Kün, 1996; Karakaya *et al*., 2016). *Hordeum bulbosum* L. is a tertiary wild relative of *H. vulgare* L. *Hordeum bulbosum* is a common species in Eastern, Southeastern and Southwestern Europe, temperate Asia, Northern Africa, United States and South America (Rhodes *et al*., 2016).

Net blotch caused by *Pyrenophora teres* Drechsler [anamorph: *Drechslera teres* (Sacc.) Shoem.] is one of the economically important diseases of barley (McLean *et al*., 2009; Liu *et al*., 2011). The disease reduces green leaf area and grain size, decreases malt quality and causes 15–35% loss of yield. Symptoms of the disease vary depending on the two different forms of fungus. *Pyrenophora teres* f. *maculata* symptoms are dark brown elliptical point necrosis and *Pyrenophora teres* f. *teres* symptoms are horizontal and vertical necrotic areas on barley leaves (Serenius *et al*., 2005; Liu *et al*., 2011). The two forms of fungus are not morphologically dissimilar, but they are symptomatically and genetically different (Bogacki *et al*., 2010; Akhavan *et al*., 2016). The fungus overwinters on the stubble and has the ability of asexual and sexual reproduction during the growth period. It is also a seed transmitted pathogen (Duczek *et al*., 1999; Karakaya *et al*., 2004). *P. teres* is a heterothallic fungus and two mating types are required for sexual reproduction (Akhavan *et al*., 2015).

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The use of fungicides and cultivation of resistant barley genotypes can control net blotch diseases. Resistance genes against net form and spot form of net blotch have been defined by different researchers (Williams *et al*., 1999; Manninen *et al*., 2000; Raman *et al*., 2003). However, plants can acquire resistance to fungicides and pathogens with high evolutionary potential can defeat the plant resistance mechanisms more (McDonald and Linde, 2002a, b). Mutation is quite important in the genetic variation of *P. teres*. Most part of life cycle of the fungus is haploid. The pathogen can propagate both sexual and asexual means and has a large evolutionary potential. Net blotch can spread to distant areas with infected seeds and *P. teres* populations may contain numerous pathotypes (Statkevičiūtė *et al*., 2010; Liu *et al*., 2011; Çelik Oğuz and Karakaya, 2017). Genetic diversity in net blotch disease has been reported by various researchers from different countries such as North America (Peever and Milgroom, 1994), Italy (Rau *et al*., 2003), and Sweden (Jonsson *et al*., 2000), Russia (Serenius *et al*., 2007), Lithuania (Statkevičiūtė *et al*., 2010), Australia and South Africa (Lehmensiek *et al*., 2010; McLean *et al*., 2010, 2014) and Western Canada (Akhavan *et al*., 2016). There are no studies related to the genetic diversity of *P. teres* in Turkey.

Inter simple sequence repeat (ISSR) marker system is a PCR-based molecular technique that provides information about the genetic relationship in the pathogen population. It is an appropriate method to evaluate the genetic structure of plant pathogens (Rampersad, 2013). In PCR, a target contains the use of a complementary primer to the microsatellite region and permits DNA amplification between microsatellite regions. This method was first described by Zietkiewicz *et al*. (1994), as a technique for genetic variation analysis in animals and plants and was subsequently used to obtain DNA markers in fungi (Bornet and Branchard, 2001; Menzies *et al*., 2003). No genomic sequence knowledge is required with ISSR-PCR and this technique can detect high levels of polymorphism. In addition, with this technique, high stability and reproducibility is possible. AFLP and RAPD markers were often used in genetic variation studies of *P. teres* (Jalli and Robinson, 2000; Jonsson *et al.*, 2000; Robinson and Mattila, 2000; Serenius *et al*., 2005; Bakonyi and Justesen, 2007). ISSR markers were used only by Statkevičiūtė *et al*. (2010) on *P. teres* f. *teres* population in Lithuania. However, there are no studies related to ISSR markers on *P. teres* f. *maculata* population. No study has been conducted regarding the genetic diversity of net blotch populations in barley growing regions of Turkey. The purpose of this study was to evaluate the genetic diversity of *P. teres* f. *maculata* and *P. teres* f. *teres* isolates obtained from cultivated barley (*Hordeum vulgare* L.) and wild barleys (*Hordeum spontaneum* C. Koch and *H. bulbosum* L.) in Turkey using ISSR genetic markers.

Materials and Methods

Isolates

Isolates were collected from different regions in Turkey in 2012–2016 and 2017. The diseased barley leaves were brought to the laboratory and after surface sterilization they were placed on moist sterile paper at 20°C for 12 h light/12 h dark for 3 days. Following the sporulation, single spores were taken under a stereo microscope and then were transferred to Petri plates containing potato dextrose agar (PDA). The majority of the single spore isolates obtained belonged to Central Anatolia and Southeast Anatolia regions of Turkey. All the isolates obtained from *H. spontaneum* and *H. bulbosum* belonged to the Southeast Anatolia region located in the Fertile Crescent region. Twenty *Ptm* and 20 *Ptt* isolates from different locations were selected from an earlier study (Çelik Oğuz *et al*., 2018). In total, 49 *P. teres* f. *maculata* and 48 *P. teres* f. *teres* isolates were used in this study. The number of isolates by region is shown in Fig. 1.

DNA Isolation

Isolates were grown on PDA medium at 22 ± 3ºC for 10 days. Cetyltrimethylammonium bromide (CTAB) method, which was modified from Allen *et al*. (2006) was used for DNA isolation. The CTAB extraction buffer (5 g of CTAB, 0.02 *M* EDTA, 1.4 *M* NaCl, 0.1 *M* Tris, pH 8.0, 0.5–1% (v/v) of β-mercaptoethanol (βME)) was added to the fungal mycelium by crushing the tissue with the MagNA lyser (Roche, Germany). The tissue then incubated at 65°C for 30 min. After centrifugation, the supernatant was transferred to new tubes containing phenol: chloroform: isoamyl alcohol (25: 24: 1). In the new centrifugation step, DNA was precipitated with cold isopropanol. Precipitated DNA was solved in Tris-EDTA (TE) buffer and treated with DNAse free RNAse-A at 37°C for 30 min. The DNA pellet was cleaned up after a series of 70% ethanol precipitation steps and then DNA was allowed to air dry. DNA was dissolved in 200 μL of pure water and stored at −20°C.

Molecular Identification and Determination of Mating Type

The PtGPD1 (control) primer pair (586 bp) was used to confirm *P. teres* (Lu *et al*., 2010). A set of single nucleotide polymorphism (SNP) primers described by Lu *et al*. (2010) were used for the distinction between net and spot forms of net blotch and determination of mating type. PCR conditions, electrophoresis and gel imaging were accomplished as described by Çelik Oğuz *et al*. (2018).

ISSR Profiling

All DNA samples were adjusted to a final concentration of

50 ng μL^{-1} in TE buffer. In this study, ten ISSR primers (UBC807, UBC810, UBC811, UBC814, UBC822, UBC825, UBC827, UBC 866, 105H, G11) were selected. These primers were previously used by Statkevičiūtė *et al*. (2010) for *P. teres* f. *teres.*

The PCR reaction mixture was carried out with 25 μL total volume containing 50 ng of fungal genomic DNA, 0.25 μM of primer, 1×Mytaq reaction buffer (15 m*M* $MgCI_2$ and 5 m*M* dNTPs) and 0.125 units of Taq DNA Polymerase (Bioline, USA). Cycling conditions were performed as followed: 95°C for 3 min, 35 cycles of 15 s at 95°C, 15 s at 50°C and 10 s at 72°C with a final extension of 72°C for 30 s.

PCR products were separated by electrophoresis on 2.5% agarose gels containing ethidium bromide with a concentration of 0.1 μg mL^{-1} and electrophoresed in 0.5 × Tris-borate-EDTA (TBE) for 3 h at 100 V. Gels were photographed under UV light (Quantum ST4, Montreal Biotech, Canada). The fragment sizes in a gel were compared with GeneRuler 100 bp DNA ladder Plus (MBI Fermentas, USA).

Data Analysis

The χ^2 significance test was used to determine the ratios of mating types observed for *P. teres* populations (Sommerhalder *et al*., 2006).

For genetic profile based on ISSR molecular data, each amplified band for each primer was scored based on the presence (1) and absence (0). The Numerical Taxonomy and Multivariate Analysis System Program package (NTSYSpc v. 2.0 (Rohlf, 1998)) based on Dice coefficient of similarity was used for calculation of genetic similarity indexes. In order to construct the dendrogram, cluster analysis of the genetic similarity matrix with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was performed.

Results

Single spore isolates were verified using species-specific- and mating type-specific PCR primers. The mating type ratios according to regions are presented in Table 1. Two mating groups (*MAT1-1* and *MAT1-2*) were found in both pathogen populations. According to the null hypothesis on sexual reproduction, *P. teres* f. *maculata* population supported the 1: 1 hypothesis with the mating type ratio, however, *P. teres* f. *teres* population deviated from 1: 1 ratio. When the regions were examined separately, *P. teres* f. *teres* population supported the 1: 1 hypothesis among the isolates from the Central Anatolian region.

In order to investigate the genetic variation of both forms of *P. teres*, 49 *Ptm* and 48 *Ptt* isolates were tested with 10 ISSR primers. Seven primers (UBC807, UBC811, UBC825, UBC827, UBC866, 105H, G11) showed optimal band numbers and good band intensities. Three primers (UBC810, UBC814, UBC822) exhibited poor and incomparable band intensities for both pathogen populations. Isolates of *Ptm* population amplified 52 bands from seven primers that ranged between 200 bp and 1400 bp. The number of ISSR fragments produced per primer varied from 5 to 11. UBC825 primer amplified the largest numbers of bands and UBC807 and UBC827 primers amplified the second most common bands for the *P. teres* f. *maculata* population.

Isolates of *Ptt* population amplified 46 bands from seven primers between 200 bp and 1100 bp. The number of ISSR fragments produced per primer varied from 4 to 11. UBC825 primer amplified the most bands and UBC807 primer followed this primer for the *Ptt* population.

Dendrograms of *Ptt* and *Ptm* populations were created with 7 primers which produced good band intensities based on the DICE similarity coefficient and UPGMA algorithm (Fig. 2 and 3).

P. teres f. *teres* isolates were divided into two main groups with a similarity of 0.51. There were 46 isolates in the first main group and two isolates in the second main group. The two isolates in the second group were obtained from different provinces in the Central Anatolia region. The first main group was divided into three groups. The majority of the first group isolates belonged to the Central Anatolia region. Two Southeast Anatolia isolates were located in this group and one Aegean isolate (t14) was separated by 0.78 similarity from the others. The majority of the second group belonged to the Southeast Anatolia region. One Marmara isolate and one Central Anatolia isolate were included in this group. In addition, t21 isolate obtained from *H. spontaneum* from the Southeast Anatolia region was included in this group. The third group isolates consisted of 3 provinces (Ankara, Eskişehir and Konya) from Central Anatolia region. The isolates from the Central Anatolia region belonged to different groups and different mating groups and showed high similarities. The isolates from different provinces of the Southeast Anatolia region with high similarity belonged to the same mating group (*MAT1-1*). The ratios of *MAT1-1* and *MAT1-2* mating types of *Ptt* population in the Central Anatolia region were close to each other whereas in the Southeast Anatolia region proportion of *MAT1-1* was higher than *MAT1-2*.

P. teres f. *maculata* isolates divided into two main groups with a similarity of 0.38. The year, location, mating type and host distribution were quite heterogeneous in both main groups. The majority of the first main group consisted of the Central Anatolia region isolates and only one Central Anatolia region isolate was in the second main group. Two of the 5 Aegean isolates were in the first group while 3 isolates were in the second group. Aegean isolates m34 and m57 were separated into different branches of the first group with approximately 0.58 similarity. The isolates m4 and m6 from the Central Anatolia region belonged to different provinces and

Regions	*P. teres* f. *maculata*			*P. teres* f. *teres*	
	Hordeum vulgare	*Hordeum spontaneum*	*Hordeum bulbosum*	*Hordeum vulgare*	*Hordeum spontaneum*
Marmara	1	-	-	1	-
Aegean	5	-	-	1	-
Southeast Anatolia	9	7	2	18	1
Central Anatolia	25	-	-	27	-
Total	40	7	2	47	1

Fig. 1: The isolates used in this study and their respective regions

different mating type groups although they had a high similarity rate. Among the seven isolates obtained from *H. spontaneum* from the Southeast Anatolia region, 5 isolates were in the first group and 2 isolates were in the second group. The genetic variation within these isolates was found to be quite high. The isolates m8 and m51, which had a high similarity rate, belonged to different groups and different mating type groups. Moreover, the isolates m26 and m29, which had a very high similarity, were from different provinces, different hosts (m26, *H. bulbosum*; m29, *H. spontaneum*) and different mating type groups. Although isolates m29, m36 (Şanlıurfa), m8, m58 (Diyarbakır) and m51 and m52 (Mardin) were obtained from the same host (*H. spontaneum*) and same provinces, they were in different groups. The isolates m31 and m26 obtained from *H. bulbosum* were obtained from different provinces located about 150 km apart from each other. These isolates were found in different mating types and in different groups of dendrograms and their genetic similarities were very low.

Discussion

Cluster analysis of two different net blotch forms using ISSR data indicated that *P. teres* f. *maculata* and *P. teres* f. *teres* populations divided into two main groups with a similarity of 0.38 and 0.51, respectively. *Ptm* population was found to be more heterogeneous than *Ptt* population with a similarity of about 0.90. Although *P. teres* f. *teres* dendogram did not clearly distinguish based on location, mating type and year, 16 isolates (84.2%) out of 19 isolates of the first group were from the Central Anatolia region. An Aegean isolate was separated alone from this group. 17 of the 19 isolates of the second group (89.4%) were Southeast Anatolia isolates. The third group consisted of completely Central Anatolia isolates.

The primers used in this study were previously tested by Statkevičiūtė *et al.* (2010) on 24 *P. teres* f. *teres* isolates collected from three different districts in different years in Lithuania. In their study, isolates were divided into 2 main groups with 0.63 similarity in the dendrogram constructed using UPGMA (DICE similarity coefficient) algorithm. There was no definite distinct location or mating type in *P. teres* f. *teres* population and all districts except Klaipeda supported 1: 1 mating type ratio. Except for our current study, there is no other study related to determination of the genetic variation using ISSR primers in *P. teres* f. *maculata* population. In our study, UBC825, UBC807 and UBC825, UBC 807, UBC827 primers generated the most polymorphic bands for *Ptt* and *Ptm* isolates, respectively. Similarly, Statkevičiūtė *et al.* (2010) indicated that the most useful ISSR primers were UBC807 and G11 for detecting polymorphism among the *Ptt* isolates. Primer G11 amplified 6 bands (450–1000 bp) in *Ptm* isolates while the same primer amplified 4 bands (450-750 bp) in *Ptt* isolates in our study. Çelik Oğuz *et al*. (2018) studied the mating types of *P. teres* in Turkey. In their study, they used 175 isolates (66 *Ptt* and 109 *Ptm*) collected between 2012–2016. *Ptm* population supported 1:1 ratio, whereas only *Ptt* Central Anatolia population supported 1:1 ratio. They emphasized that except Central Anatolia region, limited number of samples obtained from regions may be responsible for these results. *P. teres* populations used in our current study showed similar results despite the small number of isolates.

Wild barleys are both valuable sources of genetic variation and useful for different stress tolerances. In addition, they have the ability to adapt to various biotic and abiotic stress conditions (Ellis *et al*., 2000; Karakaya *et al*., 2016; Çelik and Karakaya, 2017). Nevo (1992) emphasized that *Hordeum spontaneum* could be used in barley breeding trials and wild-gene pool formation. Levant, Turkey and East of Turkey are three main regions of wild barley (*H. spontaneum*) populations (Jakob *et al*., 2014). The Southeast Anatolia region of Turkey is located in the Fertile Crescent region. In this area, barley was cultivated for the first time and has been grown for thousands of years (Kün, 1996; Brown *et al*., 2009). In our current study, one *Ptt* isolate obtained from *H. spontaneum* from Southeast Anatolia region was included in the group that consisted mainly isolates from Southeast Anatolia region. In general, *Ptm* isolates from wild barleys (*H. spontaneum* and *H. bulbosum*) did not constitute a cluster and they were placed in different groups. Two isolates, m26 (Kilis) and m29 (Şanlıurfa) obtained from different wild barley species showed 0.95 similarity. However, similarities of m29 and m36 isolates obtained from *H. spontaneum* from Şanlıurfa, were low. Similarly, isolates m8 and m51 obtained from Diyarbakır and Mardin provinces showed 80% genetic similarity and the genetic similarity of isolates m51, m54 and m58 from Mardin province were lower although they

Table 1: Mating type distribution and frequencies of *Pyrenophora teres* f. *maculata* and *Pyrenophora teres* f. *teres* populations obtained from different *Hordeum* species and different regions of Turkey

Hordeum vulgare	***P. teres* f. *maculata***		**χ^2 (1:1) types**	***P. teres* f. *teres***		**χ^2 (1:1) types**
Regions	***MAT1-1***	***MAT1-2***	**(df = 1)**	***MAT1-1***	***MAT1-2***	**(df = 1)**
Marmara	1	-	-	-	1	-
Aegean	3	2	0.2 P=0.6547	1	-	-
Southeast Anatolia	4	5	0.1111 P=0.7389	14	4	5.5556 P=0.0184*
Central Anatolia	14	11	0.36 P=0.5485	15	12	0.3333 P=0.5637
H. spontaneum* and *H. bulbosum	***MAT1-1***	***MAT1-2***		***MAT1-1***	***MAT1-2***	
Southeast Anatolia	5	4	0.1111 P=0.7389	1	-	-
Total	**27**	**22**		**31**	**17**	
χ^2 (1:1) types	**0.5102**			**4.0833**		
(d.f = 1)	**P=0.4751**			**P=0.0433***		

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

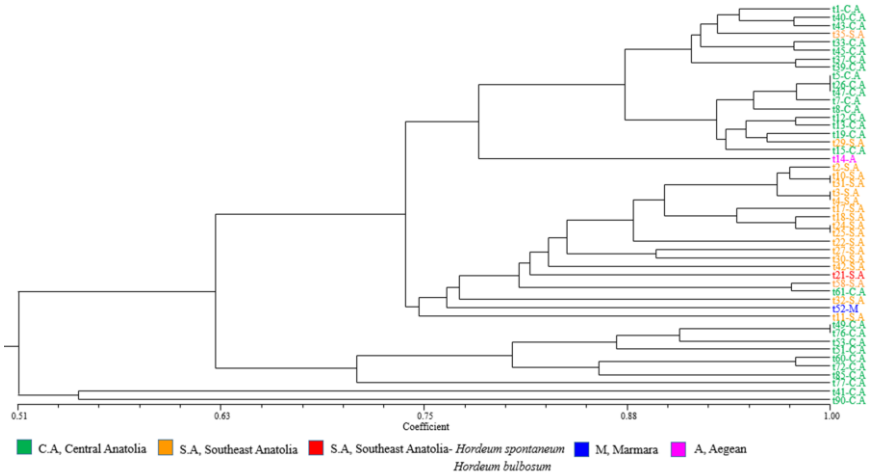

Fig. 2: Diversity of *P. teres* f. *teres* isolates obtained from different *Hordeum* species and different regions of Turkey

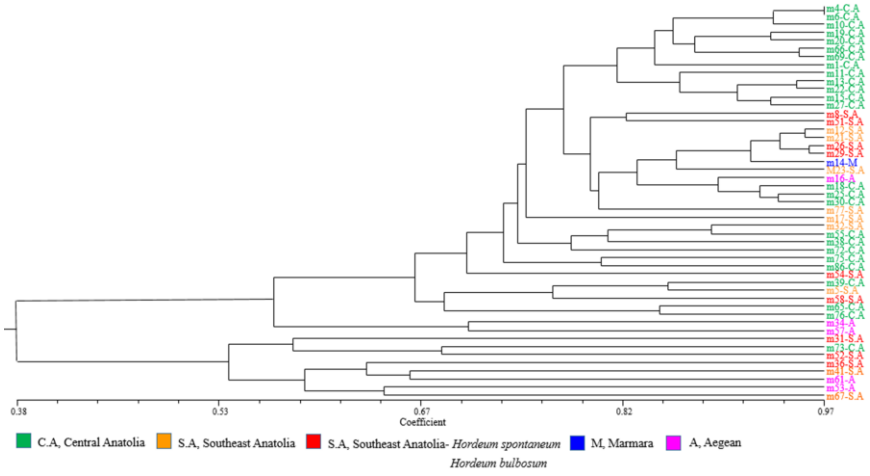

Fig. 3: Diversity of *P. teres* f. *maculata* isolates obtained from different *Hordeum* species and different regions of Turkey

were placed in the same mating group (*MAT1-2*).

In the current study, the similarity rate of *Ptm* population was found to be lower. *Ptm* population contained more haplotypes than *Ptt* population and mating type ratio of *Ptm* population did not deviate from 1:1 ratio. Considering the mating type ratio, the number

of observed high haplotypes is thought to be the result of sexual reproduction and this could generate more genotypes. High genotypic diversity was found in the population structure of *P. teres* by various researchers. Akhavan *et al*. (2016) assessed genetic variation of *Ptm* and *Ptt* populations obtained from 3 different provinces of Western Canada with 13 SSR markers and found polymorphism in both populations. In their study, mating type ratio did not deviate from 1:1 in 3 provinces of Western Canada. McLean *et al.* (2010) observed genetic variation and presence of both mating types of *Ptm* isolates in Australia. The same situation was also observed in *Ptm* and/or *Ptt* populations from Finland (Serenius *et al*., 2005, 2007), Italy (Rau *et al*., 2003), Russia, Australia (Serenius *et al*., 2007), Czechia (Leisova *et al*., 2005a, b) and Sweden (Jonsson *et al.*, 2000). However, some researchers reported that genetic variation of *P. teres* f. *teres* populations were higher than that of *P. teres* f. *maculata* populations. This was due to a lower rate of migration and the presence of asexual or sexual breeding in populations (Rau *et al*., 2003; Serenius *et al*., 2007; Lehmensiek *et al*., 2010).

Net form of net blotch is more common than spot form of net blotch in the world and numerous studies were performed with *P. teres* f. *teres* (Louw *et al*., 1996; Liu and Friesen, 2010). Serenius *et al*. (2005) found all isolates as net form in the Finnish population but spot form was more common than net form in other Scandinavian countries including Denmark (Jørgensen *et al*., 2000). Both forms were common in Western Australia (Gupta and Loughman, 2001), Italy (Rau *et al*., 2003), South Africa (Campbell *et al*., 2002) and spot form was more common in Canada (Berg and Rossnagel, 1991). Net and spot form ratios can change over the years. The reasons for these changes in the populations are unknown. *P. teres* f. *maculata* was found to be more common in Turkey (Karakaya *et al*., 2014; Çelik Oğuz *et al*., 2018). In addition, Çelik Oğuz *et al.* (2017) reported that pathotype/isolate ratio of *Ptm* was higher than that of *Ptt* in Turkey.

In this study, we did not observe any correlation related to the geographical origin of isolates. This was more obvious with *Ptm* isolates. Similarly, various researchers obtained *Ptt* and *Ptm* isolates from different geographical locations and they could not find correlation based on geographical origin of isolates (Serenius *et al*., 2005; Bakonyi and Justesen, 2007; McLean *et al*., 2010; Statkevičiūtė *et al*., 2010). When the pathogen spreads with asexual spores at long distances, the risk of epidemic induction through migration and gene flow is high (Burdon and Silk, 1997; McDonald and Linde, 2002b). *P. teres* is thought to have limited conidial spreading potential (Deadman and Cooke, 1989) but both seed-borne infections and seed movement can potentially increase gene flow (Hampton, 1980).

In our study, no differences were observed between collection years for the *Ptm* and *Ptt* populations. Akhavan *et al*. (2016) and Statkevičiūtė *et al*. (2010) reported similar findings but Leisova-Svobodova *et al*. (2014) and Liu *et al*. (2012) reported that sampling year affected diversity more than the origin of the isolates.

Monitoring of virulence levels in pathogenic populations is important for disease control. Differences may occur in the pathogen population over the years in the same geographical region. Jalli and Robinson (2000) and Robinson and Mattila (2000), using AFLP markers, reported that *Pyrenophora teres* populations mostly had asexual reproduction in Finland and genetic variation was minimal. However, using the same method, Serenius *et al*. (2005) reported a mating type ratio of *P. teres* f. *teres* in Finland population close to 1: 1 and reported high genetic variation.

When the pathogen population is small, mutations are not as important as sexual reproduction. Genotype variation is high in pathogen populations with sexual reproduction. This is one of the most important risks for the control of pathogens. Pathogens with mixed reproductive systems (sexual + asexual) are thought to have the greatest risk of evolution (McDonald and Linde, 2002b). *P. teres* populations are considered to have a mixed reproductive system in Turkey (Karakaya *et al*., 2004; Çelik Oğuz *et al*., 2018 and this study) and this could hamper control studies.

Conclusion

Genetic diversities of *Ptt* and *Ptm* populations in Turkey was determined for the first time with this study. In addition to cultivated barley, the presence of wild barley species is important for the maintenance and distribution of the pathogenic population. The isolates obtained from *H. spontaneum* and *H. bulbosum* used in our study differed in genetic similarity. The similarity rates were found as 0.38 and 0.51 in *Ptm* and *Ptt* isolates respectively and both mating types were observed in both populations. This may lead to an increase in the number of virulent pathotypes. Understanding the factors that influence pathogen evolution, monitoring pathogen biology and genetic diversity will help to develop effective control strategies against net blotch diseases of barley.

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