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Seedling Response of Iranian Barley Landraces to *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata*

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ABSTRACT

Net blotch caused by *Pyrenophora teres* is an important pathogen of barley plants worldwide. There are two biotypes of the fungus. *Pyrenophora teres* f. *teres* (*Ptt*) causes the net form of the disease and *Pyrenophora teres* f. *maculata* (*Ptm*) causes the spot form of the disease. Barley landraces are good sources of disease resistance. In this study, seedling response of 25 barley landraces obtained from different regions of northwest Iran to 3 single spore isolates of *Ptt* and 3 single spore isolates of *Ptm* were determined under greenhouse conditions. Differences in virulence among the isolates were evident. Some landraces showed different responses to different isolates. Landraces # 9 and # 16 showed moderately resistant reactions to one isolate of *Ptt* and showed moderately resistant-moderately susceptible reactions to 2 isolates of *Ptt*. Landraces # 7, # 11, # 15, # 17, # 21, # 22, # 23 and # 25 showed moderately resistant-moderately susceptible reactions to all 3 isolates of *Ptt*. Landrace # 23 showed resistant-moderately resistant reaction to one isolate of *Ptm* and showed moderately resistant reactions to 2 isolates of *Ptm*. Landrace # 16 showed moderately resistant reactions to all isolates of *Ptm*. Landraces # 11, # 15, # 21 and # 25 showed moderately resistant reaction to one isolate of *Ptm* and showed moderately resistant-moderately susceptible reactions to 2 isolates of *Ptm*. Landraces that exhibited reactions between resistant and moderately resistant-moderately susceptible range could be used as a direct seeding material to the field or could be used as breeding materials.

Keywords: *Drechslera teres*; Barley; Landrace; Net form of net blotch; Spot form of net blotch

1. Introduction

Barley (*Hordeum vulgare* L.) is the most cultivated cereal crop after wheat, rice and maize in the world (FAO 2015). It is the most planted cereal after wheat in Turkey (TUIK 2016; Geçit 2016).

Archaeological findings showed that barley was domesticated in various places of the Fertile Crescent (Zohary & Hopf 1993). Parts of Turkey and Iran are located in the Fertile Crescent region. *Hordeum spontaneum*, progenitor of cultivated barley, is also common in this region (Harlan & Zohary 1966; Nevo 1992). Eight main regions including China, India, Near East, Central Asia, Ethiopia, Mediterranean, Central and South America and Southern Mexico are considered as plant gene centers in the world (Vavilov 1951). Turkey and Iran are very important phytogeographical regions due to presence of Mediterranean as well as Central Asiatic taxa (Von Bothmer 1996). Barley landraces are still planted in these areas.

Barley plant is resistant to adverse conditions and has high adaptation capability. It can grow in various soil and climatic conditions (Mathre 1982; Geçit 2016). Wild barleys and barley landraces are new sources of genetic variation useful for different stress tolerances. They are regarded as a 'gold mine' because of their potential power to develop new genotypes against various biotic and abiotic stress factors (Yitbarek et al 1998; Ceccarelli & Grando 2000; Ellis et al 2000). Barley landraces show optimum adaptability to changing environmental conditions (Allard & Bradshaw 1964). Landraces have rich antioxidant and mineral contents and these properties can be used to develop varieties with better quality traits (Newton et al 2010). Iranian barley landraces can be considered as important gene sources for modern cultivar improvement (Khodayari et al 2012).

Net blotch is an important barley foliar disease and causes significant decreases in yield and quality of barley. Two biotypes of fungus cause different symptoms. *Pyrenophora teres* f. *maculata* (*Ptm*) incites spot type of net blotch and *P. teres*. f. *teres* (*Ptt*) incites net type of net blotch (Shipton et al 1973; Mathre 1982; Karakaya & Akyol 2006; Liu et al 2011). The prevalence of net blotch disease is related to the susceptibility of cultivated varieties. Yield losses can be 100% in severely affected fields where highly susceptible cultivars are planted. However, general losses range between 10-40% (Mathre 1982). The use of fungicides, cultural practices and planting resistant barley genotypes against the disease are recommended (McLean et al 2012). The most profitable and ecologically friendly method to control of net blotch is through using resistant barley cultivars.

In this study, we evaluated the seedling responses of 25 Iranian barley landraces obtained from different parts of Iran to 3 single spore isolates of *Ptt* and 3 single spore isolates of *Ptm* collected from different provinces of Turkey. An abstract of this study has been published previously (Çelik Oğuz et al 2017a).

2. Materials and Methods

2.1. Plant materials

Barley landraces were collected from Oshnaviye, Piranshahr, Bukan and Naghadeh regions of northwest Iran. From these, healthy looking individial seeds were selected and planted into 24 cm in diameter plastic pots filled with field soil. These pots were placed outside and watered as needed. Both light colored and dark colored seeds were selected. Seeds of these landraces were harvested after maturity and were used in this experiment.

2.2. Making single spore isolates

The infected barley leaves with net and/or spot form of net blotch were obtained from Sivas, Şanlıurfa, Kilis, Ankara, Konya and Diyarbakır provinces of Turkey. Leaf samples were cut into small pieces and surface sterilized with 1% sodium hypochloride. These pieces were incubated for 3 days on moist filter paper in sterile Petri dishes. Single spores were taken under a stereomicroscope and then transferred to the Petri dishes containing Potato Dextrose Agar (PDA).

2.3. Inoculation and incubation

No sporulation was observed in PDA, therefore, hyphal parts were used as inoculum. Previous studies showed that inoculation with hyphae was successful (Douiyssi et al 1998; Karakaya & Akyol 2006; Çelik Oğuz et al 2017b). Inoculum was prepared using 10 days old *Ptt* and *Ptm* cultures grown on PDA by brushing the culture and then filtering through cheesecloth. Mycelium particles (15-20 x 10^4 per mL) were adjusted using Thoma slides and 1 mL Tween 20 was added per 100 mL inoculum (Aktaş 1995; Douiyssi et al 1998; Karakaya & Akyol 2006; Çelik Oğuz et al 2017b). Inoculum was then sprayed onto barley leaves using a hand sprayer at the growth stages 12-13 (Zadoks et al 1974). The plants were kept in lid boxes for 76 hours under greenhouse conditions. After this period, ventilation of the boxes was opened and they were kept for 48 hours. The temperature of the greenhouse was 17±2 °C night and 22±2 °C day with a 14h/10h light/dark regime. Three replications were employed.

2.4. Disease assessment

Disease evaluations were made 7 days after inoculation using Tekauz (1985) scales which are based on lesion morphology of net blotch biotypes.

3. Results and Discussion

Twenty-five Iranian barley landraces showed different responses to 3 *Ptt* and 3 *Ptm* isolates (Table 1). Pathogenic variation was observed between *P. teres* isolates and Iranian barley landraces. Response of landraces to *Ptt* and *Ptm* isolates ranged between moderately resistant and moderately susceptible-susceptible. The most virulent isolates of *Ptt* and *Ptm* were *Ptt* 1 Sivas and *Ptm* 1 Ankara isolates, respectively.

Table 1- Seedling reactions of 25 Iranian barley landraces to 3 *Pyrenophora teres* f. *teres* and 3 *Pyrenophora teres* f. *maculata* isolates. For disease evaluation scales developed for net form of net bloth and spot form of net blotch by Tekauz (1985) were used

Landrace no	*Location*	*Row type*	*Kernel color*	*Pyrenophora teres f. teres*				*Pyrenophora teres f. maculata*			
				Ptt 1 Sivas	*Ptt 2 Şanlıurfa*	*Ptt 3 Kilis*	*Mean*	*Ptm 1 Ankara*	*Ptm 2 Konya*	*Ptm 3 Diyarbakır*	*Mean*
1	Naghadeh	2	Light	7	7	5	6.3	7	7	7	7
2	Naghadeh	2	Light	8	7	3	6	5	7	7	6.3
3	Bukan	2	Light	6	8	5	6.3	7	5	5	5.6
4	Naghadeh	2	Dark	8	6	5	6.3	8	7	5	6.6
5	Oshnaviye	2	Light	8	4	3	5	8	5	7	6.6
6	Naghadeh	2	Dark	7	4	6	5.6	7	7	7	7
7	Bukan	2	Light	5	5	5	5	5	7	5	5.6
8	Oshnaviye	2	Light	7	6	3	5.3	5	7	3	5
9	Bukan	2	Light	4	4	3	3.6	7	2	5	4.6
10	Piranshahr	2	Light	6	6	7	6.3	7	3	5	5
11	Piranshahr	6	Light	5	4	5	4.6	5	3	5	4.3
12	Naghadeh	2	Light	8	4	6	6	7	7	5	6.3
13	Naghadeh	2	Dark	8	6	6	6.6	7	7	5	6.3
14	Naghadeh	2	Dark	7	4	6	5.6	7	7	7	7
15	Naghadeh	2	Light	5	5	5	5	5	3	5	4.3
16	Bukan	2	Light	6	6	3	5	3	3	3	3
17	Piranshahr	6	Light	6	6	4	5.3	7	5	3	5
18	Oshnaviye	2	Light	6	4	7	5.6	5	5	7	5.6
19	Piranshahr	2	Light	7	5	7	6.3	7	3	7	5.6
20	Naghadeh	2	Dark	7	4	5	5.3	7	7	5	6.3
21	Naghadeh	2	Light	5	5	4	4.6	5	3	5	4.3
22	Piranshahr	2	Light	5	4	4	4.3	7	7	3	5.6
23	Naghadeh	2	Light	4	6	4	4.6	3	3	2	2.6
24	Naghadeh	2	Dark	5	7	4	5.3	7	5	3	5
25	Bukan	2	Light	4	4	4	4	5	5	3	4.3
Mean				6.16	5.24	4.76		6.12	5.2	4.96	

Fourteen, 21 and 17 landraces exhibited moderately resistant-moderately susceptible reactions to *Ptt* 1 Sivas, *Ptt* 2 Şanlıurfa and *Ptt* 3 Kilis isolates, respectively. Five landraces (# 2, # 5, # 8, # 9 and # 16) showed moderately resistant reactions to *Ptt* 3 Kilis isolate. Landraces # 7, # 11, # 15, # 17, # 21, # 22, # 23 and # 25 showed moderately resistant-moderately susceptible reactions to all 3 isolates of *Ptt*. Landraces # 9 and # 16 were moderately resistant-moderately susceptible to two isolates of *Ptt* and were moderately resistant to one isolate of *Ptt*. Eight, 6 and 11 landraces showed moderately resistant-moderately susceptible reactions to *Ptm* 1 Ankara, *Ptm* 2 Konya and *Ptm* 3 Diyarbakır isolates, respectively. Two landraces (# 16 and # 23) were moderately resistant to *Ptm* 1 Ankara isolate. In addition, 7 landraces were moderately resistant and one landrace (# 9) was resistant-moderately resistant to *Ptm* 2 Konya isolate. Six landraces were moderately resistant and one landrace (# 23) was resistant-moderately resistant to *Ptm* 3 Diyarbakır isolate. Landrace # 23 showed resistant-moderately resistant reaction to one isolate of *Ptm* and showed moderately resistant reactions to 2 isolates of *Ptm*. Landrace # 16 exhibited moderately resistant reactions to all isolates of *Ptm*. Landraces # 11, # 15, # 21 and # 25 were moderately resistant to one isolate of *Ptm* and exhibited moderately resistant-moderately susceptible reactions to 2 isolates of *Ptm*. Landrace # 9 was resistant-moderately resistant to one isolate of *Ptm* and landraces # 8, # 10, # 11, # 15, # 17, # 19, # 21, # 22 and # 24 were moderately resistant to one isolate of *Ptm*.

Fertile Crescent is the most likely geographical area where the wild barley is domesticated and wild barley populations located in the Fertile Crescent have contributed genetic material to the cultured barley (Zohary & Hoph 1993; Badr et al 2000; Morrell & Clegg 2007). This creates a large variaton in the genetic base of barley. McLean et al (2009) reported the presence of resistant genotypes among barley genotypes in the Middle East.

Barley has been grown in Fertile Crescent region a long period of time and a rich genetic diversity exist in this area (Ceccarelli & Grando 2000; Khodayari et al 2012). Ebrahimi et al (2013) investigated the genetic diversity of 115 barley landraces and wild barleys from 5 *Hordeum* species and significant variation was observed between the landraces. Khodayari et al (2012), using microsatellite markers, investigated the genetic diversity among the Iranian barley landraces and Khazaei et al (2012) characterized the agronomic traits of winter barley landraces and 4 advanced varieties collected from Iran. Both studies reported high levels of polymorphism and genetic diversity among the Iranian barley genotypes.

There are limited studies on the resistance of Iranian barley landraces to *P. teres*. Ghazvini & Tekauz (2007) tested 160 barley accessions from Iran for their reactions to *Fusarium graminearum*, *Bipolaris sorokiniana* and *Dreschlera teres* f. *teres* (teleomorph: *Pyrenophora teres* f. *teres*) in order to find new resistance sources. Three accessions were found to be resistant to *Dreschlera teres* f. *teres*. No resistance to *Fusarium graminearum* and *Bipolaris sorokiniana* isolates was found. It is concluded that disease resistant landraces were important in achieving sustainability and they were valuable sources in germplasm collections. In the current study, we identified Iranian landraces that exhibited different levels of resistance to both forms of net blotch. In current study, 5 Iranian barley landraces showed moderately resistant reactions to *Ptt* 3 Kilis isolate. Landrace number 16 exhibited moderately resistant reactions to all *Ptm* isolates and landrace number 23 showed moderately resistant reactions to 2 isolates of *Ptm* and showed resistant-moderately resistant reaction to one isolate of *Ptm*.

Large number of disease resistant barley genotypes were found in the gene centers of barley (Afanasenko et al 2000). Numerous studies reported the resistance of barley landraces to *P. teres* from different parts of the world. Lakew et al (1995) and Yitbarek et al (1998) assessed the reactions of Ethiopian barley landraces to *P. teres* and observed significant variation in landraces. Legge et al (1996) tested the resistance status of 176 Turkish barley lines to *P. teres*. More lines showed resistance to spot form of net blotch than net form of net blotch. In our study, similarly, Iranian landraces exhibited different levels of resistance to *P. teres* isolates and more Iranian landraces showed resistance to spot form of net blotch. In a study conducted by Semeane (1995) in Ethiopia only 4 of 900 barley landraces were found to be resistant to net blotch. Silvar et al (2010) tested 159 barley landraces and 16 barley cultivars from Spanish Barley Core Collection to 3 *P. teres* f. *teres* isolates. Landraces showed low resistance and only one landrace showed resistant reaction to all isolates used. Endresen et al (2011) evaluated trait-specific subset selection methods for net form of net blotch. Neupane et al (2015) evaluated 2062 barley accessions obtained from World Barley Core Collection to four *P. teres* f. *teres* isolates obtained from Australia, United States, Denmark and New Zealand. Fifteen accessions were found to be resistant to all isolates. In our study, a high number of Iranian barley landraces showed reactions in the range of resistant-moderately resistant to moderately resistant- moderately susceptible to both forms of the pathogen. Chakrabarti (1968) and Khan & Boyd (1969) tested barley varieties from World Barley Core Collection for their reactions to net blotch. In their studies, thirty of 6246 barley varieties and 6 of 8756 barley varieties were found to be very resistant, respectively. Turkey, which is located in the Fertile Crescent region, is one of the important gene centers of barley and has important barley genetic resources. Wild barley and cultivated barley landraces obtained from Turkey and Jordan were evaluated for their resistance status to *Cochliobolus sativus*, *P. teres* f. *maculata* and *P. teres* f. *teres* collected from Canada. Wild barley genotypes were found to be more resistant to *C. sativus* and *P. teres* f. *teres*. Equal amounts of wild barleys and cultivated landraces of barley were found to be resistant to *P. teres* f. *maculata* (Jana & Bailey 1995). Çelik Oğuz et al (2017b) tested 198 Turkish barley landraces to 6 virulent isolates of net form of net blotch and spot form of net blotch. 13 barley landraces showed resistant reactions to all *P. teres* f. *maculata* isolates and 7 barley landraces showed resistant reactions to all *P. teres* f. *teres* isolates. In addition, numerous landraces exhibited resistant reactions to at least one isolate. Similarly, in our current study, more Iranian barley landraces showed resistant group reactions to *Ptm* isolates than *Ptt* isolates. Several Iranian landraces were found to be resistant-moderately resistant or moderately resistant to both forms of the pathogen.

New gene resources resistant to diseases, pests and changing climatic conditions are needed for sustainable agriculture. Landraces have desirable agronomical traits and are sources of wide variation (Ceccarelli & Grando 2000; Ergün et al 2017). Useful agronomical traits could be transferred to advanced varieties successfully (Newton et al 2010). These genetic resources should be collected from natural habitats and should be protected (Frankel & Hawkes 1975).

4. Conclusions

Barley landraces are valuable sources of disease resistance. In this study, 25 barley landraces collected from different regions of northwestern Iran were tested to both forms of *P. teres* with the aim of finding sources of resistance. Fifteen of 25 landraces showed moderately resistant or resistant-moderately resistant reactions to 1 or more isolates. A wide variation was observed among the Iranian barley landraces to pathogen isolates.

Virulence changes can occur in various ways in fungi and resistant genotypes may show susceptible reactions to emerging virulent pathogens (Burdon & Silk 1997; Liu et al 2011). In order to control new pathotypes, resistance studies should be continuous and the establishment of a broad genetic base is necessary for durable and sustainable resistance. Iranian barley landraces determined in this study could be used as gene sources in future breeding studies in order to obtain net blotch resistant barley genotypes.

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