Selcuk Journal of Agriculture and Food Sciences SJAFS

http://sjafs.selcuk.edu.tr/sjafs/index

(2022) 36 (1), 20-26

e-ISSN: 2458-8377

DOI:10.15316/SJAFS.2022.004

Research Article

Determination of The Seedling Reactions of Some Two-Rowed Barley Landraces Maintained at Osman Tosun Gene Bank to Pyrenophora Teres F. Teres

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ARTICLE INFO

Article history:
Received date: 12.10.2021
Accepted date: 24.12.2021

Keywords:
Barley
Barley landraces
Drechslera teres f. *teres*
Disease resistance
Net blotch

ABSTRACT

Seedling stage reactions of thirty-eight 2-rowed barley landraces representing different areas of Turkey obtained from Osman Tosun Gene Bank to two Pyrenophora teres f. teres isolates were evaluated. In addition, barley cultivars Bülbül 89 and Avcı 2002 were included. Landraces exhibited different reactions to the disease and their reactions ranged from resistant- moderately resistant to susceptible. Landrace number 33 obtained from Diyarbakır was found the most resistant to the disease compared to all other landraces whereas landrace number 10 obtained from Bilecik-Söğüt, and cultivar Bülbül 89 were the most susceptible. The majority of the landraces were classified between the Moderately Resistant-Moderately Susceptible to Moderately Susceptible-Susceptible. Landrace number 33 exhibited Resistant-Moderately Resistant reactions to both isolates. This landrace from Diyarbakır could be used as the seed source and in the breeding studies for obtaining barley cultivars resistant to the net form of net blotch disease.

1. Introduction

Barley is one of the oldest domesticated crops and an important cereal (FAO, 2015). It is ranked as the second most important cereal in Turkey (Geçit, 2016; TUIK, 2016). Today barley is used commercially as the main source of food in livestock production, healthful diets, and in the malt industry (Kün, 1996). In Turkey, the average production of barley is estimated at 8.300.000 tonnes per year (TUIK, 2020).

Landraces are recognized for their importance as a germplasm source for barley breeding programs and for improving the genetic diversity of barley and they are adapted to stress factors (Brush, 1995; Attene *et al*., 1996). Also, barley landraces are used as the main seed source in many of the traditional barley fields by the farmers (Ceccarelli and Grando, 2000).

Landraces are well adapted to the different agroclimatic conditions, and they have emerged as a result of many years of selection. However, with the emergence of genetically uniform, high-yielding, and high-quality commercial varieties, farmers preferred to use commercial varieties. Over time, the replacement of the landraces with a high degree of variation by commercial varieties has resulted in a loss of genetic diversity (Ceccarelli *et al*., 2000; Ceccarelli and Grando, 2000). Genetic stock studies were started in Turkey in 1938 by Osman Tosun. Barley landraces have been collected by Osman Tosun and his colleagues from different parts of Turkey. Barley germplasm from several countries is also maintained in Osman Tosun Gene Bank, Ankara, Turkey (Çelik Oğuz *et al.*, 2019).

Pyrenophora teres (anamorphic stage: *Drechslera teres*) is an important pathogen of barley. Two forms of the disease, spot, and net forms exist. *Drechslera teres* f. *teres* incites the net form of the disease (Liu *et al.*, 2011). The disease affects badly the quantity and quality of barley crops worldwide. In places where very susceptible varieties are cultivated, destruction of the crops is expected (Mathre, 1982).

Both forms of the pathogen is common in Turkey (Karakaya *et al*.,2014; Damgacı, 2014; Çelik and Karakaya 2015; İlgen *et al*., 2017; Özdemir *et al*., 2017; Ertürk *et al*., 2018; Saraç *et al*., 2019; Sivrikaya *et al*., 2020). Both forms of the pathogen cause numerous races and this may complicate the control efforts. In a study conducted in Turkey, 24 *Pyrenophora teres* f. *teres* pathotypes and 26 *Pyrenophora teres* f. *maculata* pathotypes were found (Çelik Oğuz and Karakaya 2017). Morphological, pathological, and genetic variation was observed among the Turkish isolates of *P. teres* (Çelik Oğuz et al., 2014; Çelik Oğuz et al 2019a). Both mating types of fungus were found in Turkey (Çelik Oğuz *et al*.,

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2018). In Ankara, Turkey, on the leaves, left on the ground, and buried, conidia, conidiophores, and pseudothecia of the pathogen were observed. These propagules were more common on the leaves left on the ground. In cooled incubator studies, pycnidia were observed. Cooled incubator studies revealed that fungus in diseased leaves and fungal cultures survived -10°C. Apparently, the fungus can survive during the winter months under Ankara, Turkey conditions (Karakaya *et al.*, 2004).

If much focus is not taken on controlling this disease, great economic losses are expectable in areas where it forms a real threat to barley production. Various measures such as stubble destruction and the application of fungicides can control the disease. However, since plant residues are still capable of producing numerous infectious spores, a complete elimination of stubble inoculum to prevent significant infection for the next season crop is a quite difficult task (Jordan and Allen, 1984). Cultural practices alone seem ineffective for the management of the disease. Chemical fungicides are highly expensive and they are unaffordable for most farmers, reduced sensitivity of the pathogen is expectable, and a fungal population can develop resistant biotypes (Olvång, 1988). Therefore, the application of other methods such as crop rotation and using of disease-resistant barley genotypes has become necessary for the control of the pathogen (Mathre, 1982; McLean *et al.*, 2012). The use of disease-resistant barley genotypes is the most profitable and eco-friendly means of controlling the disease.

The growth of resistant cultivars is the preferred control method (Mathre, 1982; Yazıcı et al., 2015). Landraces are genetic variation sources in plant breeding programs (Yitbarek *et al*., 1998). Elucidation of the resistance status of barley landraces will be useful in breeding programs. Also, some high-yielding landraces could be used by farmers. Barley landraces can be planted either directly in the field or utilized in breeding programs for developing new resistant varieties. In Turkey, limited work exists on the net type resistance status of barley genotypes (Yazıcı *et al.*, 2015; Çelik Oğuz *et al*., 2016; 2017, 2019b).

In this work, under greenhouse conditions, 38 Turkish barley landraces obtained from Osman Tosun Gene Bank to two *Pyrenophora teres* f. *teres* isolates have been evaluated at the seedling stage. In addition, two barley cultivars Bülbül 89 and Avcı 2002 were included in this study.

2. Materials and Methods

This study was accomplished in the laboratory and the greenhouse of the Ankara University, Faculty of Agriculture, Department of Plant Protection, Turkey. All trials in the greenhouse were executed as three replications. A total of 38 barley landraces were used in the study. The landraces used in this study were obtained from Osman Tosun Gene Bank, Ankara, Turkey. In addition, barley cultivars Bülbül 89 and Avcı 2002 were included in this study. The locations of the barley landraces were presented in Figure 2.

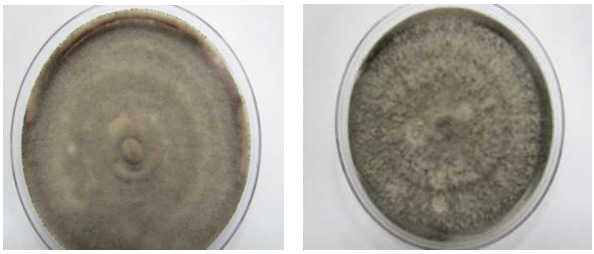

Figure 1
Single spore culture of Ankara isolate in Potato Dextrose Agar medium (PDA) (left), single spore culture of Sivas isolate in PDA medium (right).

Inoculation was accomplished using 2 *Pyrenophora teres* f. *teres* isolates obtained from the Ankara and Sivas provinces of Turkey (Figure 1). Isolates were obtained from Aziz Karakaya and Arzu Çelik Oğuz, Ankara University, Turkey.

The Gene Bank Registration numbers of the thirty-eight landraces used in the study, the locations from which they were obtained, and the color of their kernels are shown in Table 1

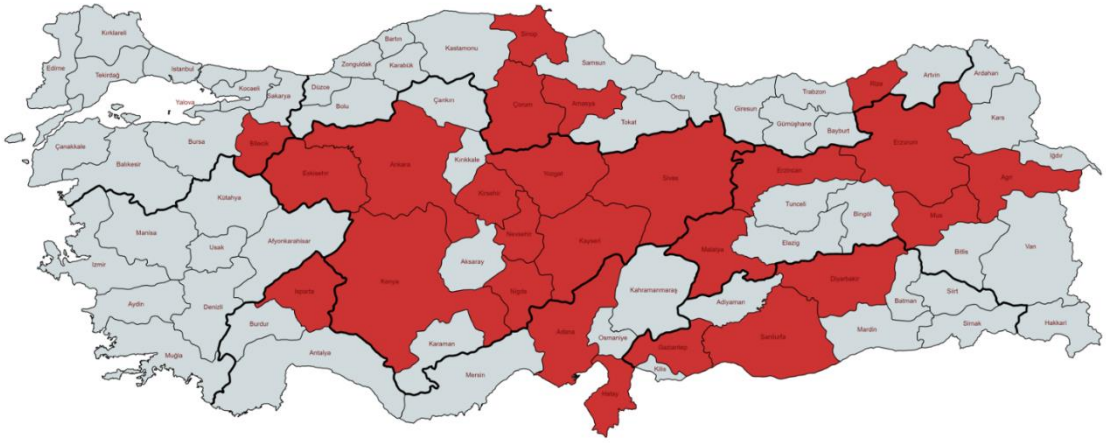

Figure 2
Provinces that barley landraces are obtained.

Table 1
Some characteristics of the landraces used in this study

No	Osman Tosun Gene Bank Registration No	Type	Location	Kernel color
1	667	2-rowed	Eskişehir-Çifteler	Black
2	885	2-rowed	Diyarbakır-Bismil	White
3	930	2-rowed	Diyarbakır-Karabaş	White
4	900	2-rowed	Diyarbakır-Nusaybin	Black
5	933	2-rowed	Erzincan-Refahiye	White
6	715	2-rowed	Kayseri-Pınarbaşı	White
7	884	2-rowed	Urfa	White
8	684	2-rowed	Ağrı-Tutak	White
9	692	2-rowed	Ankara	White
10	659	2-rowed	Bilecik-Söğüt	White
11	638	2-rowed	Rize-Pazar	White
12	868	2-rowed	Konya-Zıvarak	White
13	671	2-rowed	Çorum-Mecitözü	White
14	709	2-rowed	Sivas-Zara	White
15	707	2-rowed	Niğde-Aksaray	White
16	702	2-rowed	Kırşehir-Kaman	White
17	799	2-rowed	Adana-Kadirli	White
18	854	2-rowed	Nevşehir	White
19	931	2-rowed	Malatya-Pötürge	White
20	914	2-rowed	Erzurum-H.Kale	White
21	825	2-rowed	Kayseri-Pınarbaşı	White
22	707	2-rowed	Malazgirt	White
23	660	2-rowed	Sinop-Gerze	White
24	685	2-rowed	Hatay-Kırıkhan	White
25	644	2-rowed	Gaziantep-Nur.	White
26	664	2-rowed	Isparta	White
27	844	2-rowed	Yozgat-Akdağmadeni	Black
28	771	2-rowed	Konya	Black
29	926	2-rowed	Urfa-Siverek	White
30	915	2-rowed	Sivas-Divriği	Black
31	640	2-rowed	Amasya-Taşova	White
32	673	2-rowed	Isparta-Gelendost	White
33	941	2-rowed	Diyarbakır	White
34	786	2-rowed	Hatay-Korkuteli	White
35	906	2-rowed	Konya-Ereğli	White
36	888	2-rowed	Eskişehir-Ağapınar	White
37	752	2-rowed	Niğde-Bor	White
38	689	2-rowed	Erzurum	White

The inoculation procedure was similar as described by several other investigators (Çelik Oğuz et al., 2019b; Yazıcı et al., 2015; Douiyssi et al., 1998). Fifteen seeds from each landrace were seeded in 7 cm diameter plastic pots containing soil. Plants were watered as necessary. The temperature of the greenhouse was 18-23±2 ºC for night and day with a 14h/10h light/dark regime. For inoculum production, mycelia were scraped from Petri plates using a paintbrush. Inoculum concentration was

adjusted using a hemocytometer to 15-20x10^4 mycelial parts per ml. One drop of Tween 20 was added for every 100 ml of the inoculum (Aktaş, 1995). After inoculation, plants were placed in metal boxes and a plastic cover was placed on top of each box. In addition, boxes and plastic covers were wrapped with nylon sheets. Plants were inoculated at growth stages 12-13 (Zadoks et al., 1974). After four days nylon sheets and plastic covers were removed. Seven days after inoculation, plants were evaluated with a 1-10 scale developed for *D. teres* f. *teres* by Tekauz (1985). The description of the scale is as follows:

1: R (Resistant),

2: R-MR (Resistant-Moderately Resistant),

3: MR (Moderately Resistant),

4:MR-MS: (Moderately Resistant-Moderately Susceptible),

5:MR-MS (Moderately Resistant-Moderately Susceptible),

6:MR-MS (Moderately Resistant-Moderately Susceptible),

7: MS (Moderately Susceptible),

8: MS-S (Moderately Susceptible-Susceptible),

9: S (Susceptible),

10: VS (Very Susceptible).

A visual scale is presented in Figure 3.

3. Results and Discussion

Ankara and Sivas isolates differed in the nature of their growth on the PDA medium. The single spore culture of Sivas isolate appeared smooth whereas Ankara isolate had fluffy growth (Figure 1).

The first symptoms of the disease were observed on leaves of some landraces three days after inoculation. Leaf symptoms characteristic of the net form of the disease appeared first as narrow chlorotic lesions which gradually increased in size and length. Eventually, severely infected leaves showed dark brown longitudinal and transverse striations (Figure 4).

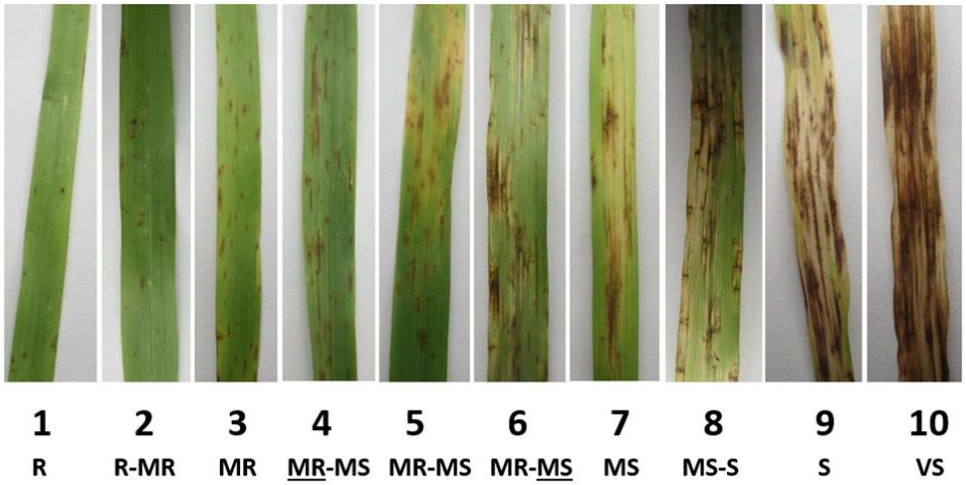

Figure 3
Visual scales used in the experiment according to Tekauz (1985) (Photographs: Aziz Karakaya).

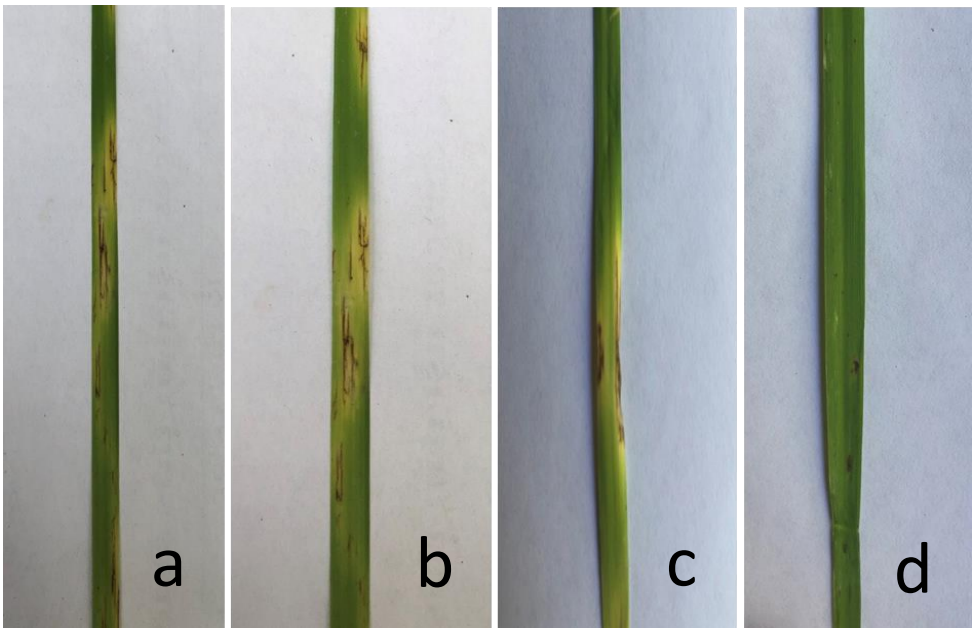

Figure 4
Leaves of barley landraces showing different levels of resistance to *Pyrenophora teres* f. *teres* Ankara isolate. a) Şanlıurfa landrace, scale value: 6 (MR-MS), b) Çorum-Mecitözü landrace, scale value: 7 (MS), c)Kayseri-Pınarbaşı landrace, scale value: 6 (MR-MS), d) Diyarbakır landrace, scale value: 2 (R-MR) (Photographs: Hatice Sevde Yüceler)

Evaluations were performed 7 days following inoculation. Results are presented in Table 2 in addition to some information concerning the 2- rowed barley landraces used in this study. Data on disease severity for each landrace are the mean scale values of the three replicates.

Table 2
Some characteristics of the barley landraces used in this study and the response of these landraces to 2 *P. teres* f. *teres* isolates at the seedling stage

No	Osman Tosun Gene Bank Registration No	Location	Sivas isolate scale value and reaction type (Tekauz 1985)	Ankara isolate scale value and reaction type (Tekauz 1985)
1	667	Eskişehir-Çifteler	5 MR-MS	5 MR-MS
2	885	Diyarbakır-Bismil	8 MS-S	8 MS-S
3	930	Diyarbakır-Karabaş	6 MR-MS	6 MR-MS
4	900	Diyarbakır-Nusaybin	6 MR-MS	5 MR-MS
5	933	Erzincan-Refahiye	7 MS	6 MR-MS
6	715	Kayseri-Pınarbaşı	7 MS	6 MR-MS
7	884	Şanlıurfa	7 MS	6 MR-MS
8	684	Ağrı-Tutak	6 MR-MS	6 MR-MS
9	692	Ankara	6 MR-MS	5 MR-MS
10	659	Bilecik-Söğüt	9 S	8 MS-S
11	638	Rize-Pazar	7 MS	6 MR-MS
12	868	Konya-Zıvarak	7 MS	7 MS
13	671	Çorum-Mecitözü	7 MS	7 MS
14	709	Sivas-Zara	8 MS-S	7 MS
15	707	Niğde-Aksaray	5 MR-MS	6 MR-MS
16	702	Kırşehir-Kaman	7 MS	6 MR-MS
17	799	Adana-Kadirli	7 MS	7 MS
18	854	Nevşehir	8 MS-S	7 MS
19	931	Malatya-Pötürge	7 MS	7 MS
20	914	Erzurum-H.Kale	6 MR-MS	6 MR-MS
21	825	Kayseri-Pınarbaşı	6 MR-MS	6 MR-MS
22	707	Muş-Malazgirt	5 MR-MS	5 MR-MS
23	660	Sinop-Gerze	5 MR-MS	5 MR-MS
24	685	Hatay-Kırıkhan	5 MR-MS	6 MR-MS
25	644	Gaziantep-Nur	7 MS	7 MS
26	664	Isparta	7 MS	7 MS
27	844	Yozgat-Akdağmadeni	7 MS	7 MS
28	771	Konya	7 MS	7 MS
29	926	Urfa-Siverek	7 MS	6 MR-MS
30	915	Sivas-Divriği	7 MS	7 MS
31	640	Amasya-Taşova	5 MR-MS	5 MR-MS
32	673	Isparta-Gelendost	7 MS	6 MR-MS
33	941	Diyarbakır	2 R-MR	2 R-MR
34	786	Hatay-Korkuteli	8 MS-S	7 MS
35	906	Konya-Ereğli	6 MR-MS	6 MR-MS
36	888	Eskişehir-Ağapınar	6 MR-MS	6 MR-MS
37	752	Niğde-Bor	7 MS	6 MR-MS
38	689	Erzurum	8 MS-S	8 MS-S
39	89	Bülbül 89	9 S	8 MS-S
40		Avcı 2002	4 MR-MS	5 MR-MS
Isolate means			6.525	6.225

Landrace reactions to Sivas isolate ranged between Resistant-Moderately Resistant and Susceptible. Landrace number 33 obtained from Diyarbakır had a 2 scale value and this landrace was rated Resistant-Moderately Resistant to the disease. Fourteen other landraces exhibited Moderately Resistant-Moderately Susceptible disease reaction. These include landraces with the numbers 1,3,4,8,9,15,20,21,22,23,24,31,35, and 36. The rest of the landraces showed moderately susceptible (MS) to moderately susceptible-susceptible (MS-S) disease responses except for the landrace obtained from Bilecik-Söğüt. This landrace showed a susceptible (S) reaction (scale value 9). Cultivar Bülbül 89 showed a scale value of 9 and cultivar Avcı 2002 showed a scale value of 4. Responses of the landraces to Ankara isolate ranged between Resistant-Moderately Resistant and Moderately Susceptible-Susceptible. Again the landrace number 33 from Diyarbakır was found Resistant-Moderately Resistant (R-MR). Landraces 1, 3, 4, 5, 6, 7, 8, 9, 11, 15, 16, 20, 21, 22, 23, 24, 29, 31, 32, 35, 36, 37 exhibited Moderately Resistant-Moderately Susceptible (MR-MS) reaction to Ankara isolate. Other landraces showed Moderately Susceptible (MS) to Moderately Susceptible-Susceptible (MS-S) reactions. Cultivar Bülbül 89

showed a scale value of 8 and cultivar Avcı 2002 showed a scale value of 5. Sivas isolate appeared to be slightly more virulent (Isolate means: Sivas isolate 6.525, Ankara isolate 6.225).

4. Discussion And Conclusion

Results of the present study indicated that reactions of the landraces tested against two *P. teres* f. *teres* isolate ranged between R-MR to S. Among the thirty-eight landraces screened in this study, only one landrace from Diyarbakır exhibited R-MR response to the isolates (scale value: 2). Fourteen and 22 landraces exhibited MR-MS responses to Sivas and Ankara isolates, respectively. The other landraces exhibited susceptible group reactions to the isolates.

Çelik Oğuz et al. (2017), using three *P. teres* f. *teres* isolates, tested 198 landraces of barley. The researchers reported that among the total number of landraces, seven landraces were resistant.

In another study conducted in 2019 by Çelik Oğuz *et al*., responses of seedlings of 25 barley landraces obtained from Iran against 3 isolates of *P. teres* f. *teres* under a controlled environment were determined. Different degrees of virulence were found among the isolates. Two landraces exhibited MR reactions to one of the *Ptt* isolates.

Legge *et al*., (1996) evaluated the disease reactions of 176 Turkish barley accessions to barley pathogens prevalent in Canada. A small number of accessions with resistance to *P. teres* f. *teres* were identified.

Barley landraces represent a wide genetic variation for desirable agronomic characteristics (Ergün *et al*., 2017), and successful transfer of desired agronomical traits with specific genes to new varieties of crops is possible (Newton *et al*., 2010). In addition, emphasis on the collection of those genetic resources from their natural range or habitat and also their conservation should be undertaken (Frankel and Hawkes, 1975).

In addition to the barley landraces, wild barley (*Hordeum spontaneum*) is also a valuable resistance source. Çelik Oğuz *et al*., (2019c) screened 104 *H. spontaneum* genotypes using virulent *Pyrenophora teres* f. *teres* isolates. Eight *H. spontaneum* genotypes showed resistant group reactions to 3 virulent isolates of *P. teres* f. *teres*.

Mutation breeding is also used to obtain disease-resistant lines. In a study conducted by Çelik Oğuz *et al*., (2016), barley cultivar Tokak 157/37 was subjected to gamma irradiation and mutant lines were obtained. Twenty-five mutant barley lines obtained by gamma irradiation were These lines were tested for their seedling resistance status under greenhouse conditions using *Drechslera teres* f. *teres* isolates. Isolate differences were evident. The reactions of the mutant lines to the most virulent isolate differed between moderately resistant-moderately susceptible and susceptible.

This current study showed the variation in barley landraces obtained from Osman Tosun Gene Bank in Turkey to *P. teres* f. *teres*. Landraces with good resistance such as the one obtained from Diyarbakır (Osman Tosun Gene Bank Registration Number 941) could be employed as a source of resistance genes in barley breeding programs.

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