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Determination of Seedling Reactions of Some Barley Cultivars, Lines, and Wild Barley (*Hordeum spontaneum*) Genotypes to *Cochliobolus* Leaf Spot Disease

DNursena Kızıl¹, DAziz Karakaya^{1,*}, DArzu Çelik Oğuz¹, DNamuk Ergün²

¹ Ankara University, Faculty of Agriculture, Department of Plant Protection, Ankara, Türkiye ² Central Research Institute for Field Crops, Ankara, Türkiye

HIGHLIGHTS

- *Cochliobolus* leaf spot disease is an important disease of barley caused by the fungus *Cochliobolus* sativus.
- Under greenhouse conditions, seedling stage reactions of 36 barley cultivars, 35 advanced lines, and 21 wild barley (*Hordeum spontaneum*) genotypes were determined.
- Among barley cultivars and genotypes, six genotypes showed a low reaction, 29 genotypes showed moderate reactions and 36 genotypes showed a high reaction.
- Of the wild barley genotypes, two, 8, and 11 genotypes showed low, moderate, and high reactions respectively.

Abstract

*Cochliobolus*leaf spot disease, also seen in barley, is an important disease caused by the fungus, *Cochliobolus sativus*. In this study, under greenhouse conditions, seedling stagereactions of 36 barley cultivars, 35 advancedlines, and 21 wild barley (*Hordeum spontaneum*) genotypes obtained from Elazığ province of Türkiye and Güzelyurt district of the Turkish Republic of Northern Cyprus against a virulent isolate of *Cochliobolus sativus* has been determined. Among barley cultivars and genotypes, six genotypes showed a low reaction, 29 genotypes showed moderate reactions and 36 genotypes showed a high reaction. Of the wild barley genotypes, two, 8, and 11 genotypes showed low, moderate, and high reactions respectively. Barley cultivars Harman and Pinar showed low reaction responses, cultivars Akar, Bolayır, Bozlak, Burgaz, Çumra 2001, Hasat, Misket, Orza 96, Sabribey, Sinanbey, Yaprak, and Yesevi 93 exhibited intermediate reaction responses and cultivars Anka-06, Asil, Avci 2002, Aydanhanım, Ayranci, Başgül, Burakbey, Bülbül 89, Cacabey, Cirit, Çetin 2000, Hazar, İnce 04, Keykubat, Larende, Özdemir 05, Sayım 40, Tarm 92, Tosunpaşa, Yalın, Yüksel, and Zeynelağa showed high reaction responses. Barley cultivars exhibiting low reaction types can be used by farmers in the field, while barley lines and wild barley genotypes showing low reactions can be used in breeding studies.

Keywords: Barley; Bipolaris sorokiniana; Cochliobolus sativus; spot blotch; disease resistance

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*Correspondence: <u>karakaya@agri.ankara.edu.tr</u>

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1. Introduction

Barley (*HordeumvulgareL.*), a member of the Poaceae family, is an annual long-day plant among the cool climate cereals. Cultivated barleys are typically categorized as 2 rowed or 6 rowed based on ear structure (Geçit 2016), and they are divided into hulled or hulless (naked) according to the separation of grains and husks after harvest (Duan et al. 2015). Barley, which can be grown without irrigation in arid and semi-arid regions, shows better growth in areas with high relative humidity, and moderate climates. *Hordeum spontaneum* C. Koch, which is accepted as the ancestor of modern barley, is two-rowed and predominantly self-pollinated (Zohary and Hopf 2000).

It is estimated that the barley cultivation areas in the world are 51.7 million ha with an increase of 1% in the cultivation areas in the 2020/21 season, and the barley production is 159.7 million tons with an increase of 1.9%. Analysis of the 2019/20 USDA datashowed that the EU has the largest barley cultivation area in the world with 21.7% in the last seven production seasons. The EU is followed by Russia, Australia, Türkiye, and Canada with 16.4%, 7.9%, 7.4%, and 5.3%. These countries constitute approximately 58.7% of the world's cultivation area (Eğilmez 2021; USDA 2022).

Approximately 55-60% of the world's barley production is used for animal feeding, 30-40% in the malt industry, 2-3% in human consumption, and 5% as seed (Ullrich 2011). Barley, which is mostly cultivated under rainfed conditions and in the arid climate zone, is a grain known for its earliness, which has an escape mechanism from drought in Türkiye (Anonymous B 2020). Barley, which ranks second in Türkiye after wheat, is produced in every agro-ecological zones of Türkiye. Barley cultivation areas and production amounts for the last ten years vary between 2.4-3.0 million hectares (ha) and 6.3-8.0 million tons, respectively (Anonymous A 2020; Eğilmez 2021; Anonymous 2021).

Cochliobolus sativus (Ito and Kuribayashi) Drechs. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem.) is one of the fungal diseases that affect barley production and quality. This pathogen, which causes *Cochliobolus* leaf spot and root rot diseases in the plant (Mathre 1997; Kumar et al.2002), is an important disease agent that leads to a decrease in quality and yield in barley. The disease was more common in barley than in wheat in the central Anatolian Region, and it was determined that one-third of the cultivation areas were contaminated with the pathogen (Aktaş 1982). *Cochliobolus* leaf spot disease is usually seen in humid conditions (Fetch and Steffenson 1999). On the leaves of the plant infected with this disease, the first symptoms are dark chocolate-colored spots and over time they form irregular necrotic patches on the leaf. As the infection progresses more severely, the infected leaf dries up completely (Mathre 1997).

Cochliobolus sativus passes to the next year with seeds and diseased plant residues in the soil and can cause disease in all development stages of the plant. Root and root collar disease symptoms caused by the fungus are dark and pale brown spots. Grain deaths occur due to reasons such as spots on leaves, seedling blight, and root rot; the husk and grain blight seen on the spike causes the embryo to darken (Yıldırım et al. 2016).

This disease causes 16% to 33% product loss with the effect of environmental conditions (Wilcoxson et al. 1990). Overall, the degree of resistance in modern cultivars is still insufficient (Hetzler et al. 1991; Chang et al. 1998; Mujeeb-Kazi 1998; Van Ginkel and Rajaram 1998; Kumar et al.2002).

Although fungicide applications are applied in the fight against this disease, the development and use of lines and varieties resistant to *C. sativus* stand out as an environmentally friendly control method (Kiesling1985). The reactions of the newly developed barley cultivars should be determined. The resistance status of advanced barley lines and wild barley (*Hordeum spontaneum*) genotypes which are important resistance sources should be assessed. *Hordeum spontaneum*genotypes are useful disease-resistance sources (Çelik and Karakaya 2017).

There are studies of resistance to diseases related to *Cochliobolus* leaf spot in the world and in Türkiye (Aktaş and Tunalı 1994; Clear et al. 1997; Legzdina and Buerstmayr 2004; Gerlegiz et al. 2015; Çelik Oğuz et al. 2016; Celik-Oguz and Karakaya 2017; Balcı et al. 2018). On a worldwide scale, yield losses in wheat and barley caused by *C. sativus* reveal the need to search for alternative strategies to combat the disease (Yıldırım 2016).

Several studies have identified quantitative trait loci (QTL) for resistance to *Cochliobolus* leaf spot disease on all seven barley chromosomes. To date, three resistance genes (Rcs 5, Rcs 6, Rbs 7) have been mapped in detail(Zhou and Steffenson 2013; Afanesenko et al. 2015; Novakaziet al. 2020; Visioni et al. 2020). In addition, common QTLs were detected in all barley chromosomes at seedling and adult plant stages (Visioni et al. 2003; Yun et al. 2020). Wild barley is an important source of genetic variation for disease resistance (Fetch et al. 2003; Yun et al. 2005). Although there are some studies on genetic resistance to *Cochliobolus* leaf spot disease, there are limited genetic studies from the wild barley host. Wild barley accession PI 466423, resistant to Fusarium head blight and *C. sativus*, was crossed with the Rasmusson cultivar, and four resistance QTLs were identified on chromosomes 1H, 2H, 4H, and 5H.These results confirm the value of using wild relatives as a source of new resistance alleles (Haas et al. 2016).

In this study, seedling stage reactions of old and new barley cultivars, advanced barley breeding materials, and wild barley (*Hordeum spontaneum*) genotypes against *C. sativus* leaf spot disease were determined.

2. Materials and Methods

In this study, seedling resistance of 36 old and newly bred barley cultivars, 35 advanced barleylines and 21 wild barley (Hordeum spontaneum) genotypes were evaluated under greenhouse conditions against a virulent isolate of Cochliobolus sativus. A highly virulent isolate obtained from Yozgat in 2015, which was stored in the culture collection of Ankara University, Faculty of Agriculture, Department of Plant Protection, Mycology laboratory, was used. Before the study, the virulence level of the isolate was tested on the susceptible variety Bülbül 89 (Celik-Oguz and Karakaya 2017; Çelik Oğuz et al. 2019), and the virulence level was again found to be high. Wild barley genotypes were obtained from Elazığ province of Türkiye (16 genotypes) and Güzelyurt district of the Turkish Republic of Northern Cyprus (TRNC) (5 genotypes). For this purpose, the methods outlined by Celik-Oguz and Karakaya (2017) were used. Reproduction of pure wild barley seeds under field conditions was carried out in the experimental field of the Central Research Institutefor Field Crops in Yenimahalle, Ankara, Türkiye. Fifteen seeds of each barley and wild barley genotype were planted in pots containing soil, sand, and organic matter (60: 20: 20, v:v:v). Conidia suspension was prepared from 10-day-old cultures grown on Potato Dextrose Agar. To prepare the inoculum, the conidia of the fungus grown in Petri dishes for 14 days were scraped with the help of a brush, filtered through cheesecloth and the density was determined using a Thoma slide as 2x10⁴ conidia/ml. 1 drop of Tween 20 was added to each 100 ml (Çelik Oğuz et al. 2016). Cultures were incubated at 20-23°C in 12 hours dark/12 hours light conditions. The plants in each pot were sprayed homogeneously with the inoculum. Inoculation was carried out at Zadoks 12-13 growth stagewhich corresponds to the 2-2.5 leaf stage (Zadoks et al. 1974). The studies were carried out under greenhouse conditions. Following inoculation, the plants were placed in boxes with transparent lids and closed with a polyethylenebag. Experiments were carried out as 3 replications. After 7 days, evaluations were made according to the 1-9 scale developed by Fetch and Steffenson (1999). This scale is divided into 3 categories. Scale values 1-3, 4-5, and 6-9 represented low, intermediate, and high infection responses, respectively.

3. Results

Seedling stage reactions of 36 barley cultivars, 35 barley lines, and 21 wild barley (*Hordeum spontaneum*) genotypes against *C. sativus* were determined (Tables 1, 2, 3 and Figures 1, 2).

Among barley cultivars and lines, six genotypes showed a low reaction, 29 genotypes showed intermediatereactions and 36 genotypes showed a high reaction. Of the wild barley genotypes, two, 8, and 11 genotypes showed low, intermediate, and high reactions, respectively. Barley cultivars Harman and Pinar showed low reaction responses; Akar, Bolayir, Bozlak, Burgaz, Çumra 2001, Hasat, Misket, Orza 96, Sabribey, Sinanbey, Yaprak, and Yesevi 93 exhibited intermediate reaction responses and cultivars Anka-06, Asil, Avci 2002, Aydanhanim, Ayranci, Başgül, Burakbey, Bülbül 89, Cacabey, Cirit, Çetin 2000, Hazar, İnce 04, Keykubat, Larende, Özdemir 05, Sayim 40, Tarm 92, Tosunpaşa, Yalın, Yüksel, and Zeynelağa showed high reaction responses.

Barley line IKABVD Ç-23 (IR 7.33) and wild barley genotype *H. spontaneum* 24 (IR 6.67) and barley cultivar Ayranci (IR 7.33) showed the most susceptible reactions against *C. sativus*. The most resistant reaction against the disease was exhibited by the *H. spontaneum* 30 (IR 2) genotype (Tables 1, 2, and 3).

Out of 21 genotypes of wild barley (*H. spontaneum*), 9.52%, 38.1% and 52.38% showed low, intermediate, and high infection responses, respectively. Among the barley cultivars, 5.56%, 33.33%, and 61.11% showed low, medium, and high infection responses, respectively. 11.43% of barley lines showed low infection response, 48.57% were placed in the intermediate group and 40% exhibited high infection values. Of the 36 barley cultivars, 35 barley lines and 21 wild barley (*H. spontaneum*) genotypes, 8.70%, 40.22%, and 51.09% were placed in the low (resistant), intermediate, and high (susceptible) infection classes, respectively.

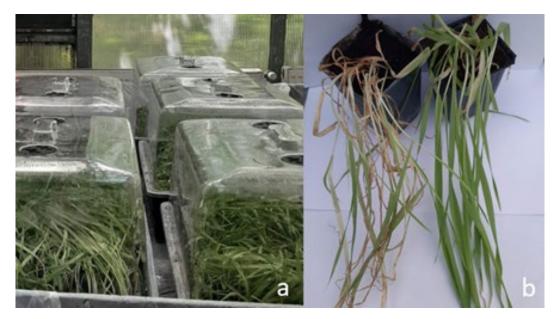


Figure 1. a) Barley genotypes after inoculation with a virulent strain of *Cochliobolus sativus* under greenhouse conditions b) Ayrancı (left) and Pınar (right) barley cultivars 7 days after inoculation

Barley Lines	Replications	Mean	Infection Classe
	3		
Std. Çeş50	3	3.33	А
	4		
	6		
Std. Çeş63	6	6	С
	6	0	
	3		
Std. Çeş64	3	3	А
	3		
	3		
Std. Çeş65	4	3.67	В
	4		
	6		
Std. Çeş67	6	5.67	С
	5		
	6		
Std. Çeş125	6	6	С
	6		
	5		
Std. Çeş126	6	5.33	В
	5		
	5		
Std. Çeş127	5	4.67	В
	4		
	5		
Std. Çeş128	5	5	В
2 2	5		
	7		
Std. Çeş133	6	6.67	С
2 2	7		
	5		
Std. Çeş145	5	4.67	В
- د د	4		
	5		
Std. Çeş146	5	5	В
	5	-	_
	5		
Std. Çeş147	5	5	В
	5	0	D
	4		
Std. Çeş148	3	3.33	А
	3	0.00	Л

Table 1. Seedling stage reactions of 35 barley genotypes against *Cochliobolus sativus* leaf spot disease.

Barley Lines	Replications	Mean	Infection Class
	5		
Std. Çeş149	5	5	В
	5		
	3		
Std. Çeş150	3	3.33	А
	4		
	5		
Std. Çeş151	5	5.33	В
	6		
	6		
Std. Çeş152	6	6	С
	6		
	7		
İKABVD Ç-2	6	6.33	С
	6		
	5		
İKABVD Ç-3	4	4.33	В
	4		
	4		
İKABVD Ç-4	4	4	В
	4		
	5		
İKABVD Ç-6	6	5.33	В
	5		
	7		
İKABVD Ç-7	7	6.67	С
	6		
	7		
İKABVD Ç-8	7	6.67	С
	6		
	6		
İKABVD Ç-9	5	5.67	С
	6		
	4		
İKABVD Ç-11	5	4.67	В
	5		
	7		
İKABVD Ç-12	6	6.67	С
	7		
	7		
İKABVD Ç-13	6	6.33	С
3	6		

 Table 1 (Continued). Seedling stage reactions of 35 barley genotypes against Cochliobolus sativus leaf spot disease (continued).

Barley Lines	Replications	Mean	Infection Classes
	5		
İKABVD Ç-14	5	5	В
	5		
	6		
İKABVD Ç-17	6	5.67	С
	5		
	7		
İKABVD Ç-18	6	6.33	С
	6		
	5		
İKABVD Ç-19	5	4.67	В
	4		
	5		
İKABVD Ç-21	6	5.33	В
	5		
	5		
İKABVD Ç-22	4	4.33	В
	4		
	8		
İKABVD Ç-23	7	7.33	С
	7		
	5.34		
General Mean	5.2	5.21	В
	5.09		

 Table 1 (Continued). Seedling stage reactions of 35 barley genotypes against Cochliobolus sativus leaf spot disease (continued)

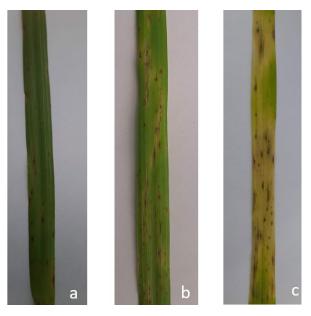


Figure 2. Infection classes showing a) low infection response b) intermediate infection response c) high infection response

Barley Cultivars	Replications	Mean	Infection Classe
	5		
Akar	5	5	В
	5		
	7		
Anka-06	7	7	С
	7		
	7		
Asil	7	7	С
	7		
	7		
Avci 2002	6	6.67	С
	7		
	6		
Aydanhanım	6	6.33	С
-	7		
	7		
Ayrancı	7	7.33	С
-	8		
	7		
Başgül	6	6.67	С
	7		
	5		
Bolayır	5	5	В
2	5		
	6		
Bozlak	6	5.33	В
	4		
	5		
Burakbey	6	5.67	С
5	6		
	5		
Burgaz	5	5.33	В
0	6		
	7		
Bülbül-89	7	6.67	С
	6		-
	7		
Cacabey	7	6.67	С
cace cy	6	0.07	C
	6		
Cirit	6	5.67	С
Clift	5	5.07	C

Table 2. Seedling stage reactions of 36 barley cultivars against Cochliobolus sativus leaf spot disease

Barley Cultivars	Replications	Mean	Infection Classe
	7		
Çetin 2000	6	6.33	С
	6		
	4		
Çumra 2001	5	4.67	В
	5		
	4		
Harman	3	3.33	А
	3		
TT .	4		P
Hasat	5	4.67	В
	5		
TT	6		C
Hazar	5	5.67	С
	6		
İnce 04	6	6	C
Ince 04	6	6	С
	6		
Keykubat	6	6	С
Кеукира	6 6	6	C
	6		
Larende	5	5.67	С
Latende	6	5.07	C
	5		
Misket	5	5	В
WINKet	5	0	D
	5		
Orza-96	4	4.33	В
	4	100	2
	6		
Özdemir 05	5	5.67	С
	6		2
	3		
Pınar	2	2.67	А
	3		
	4		
Sabribey	5	4.67	В
5	5		
	6		
Sayım 40	6	6	С
2	6		

 Table 2 (Continued). Seedling stage reactions of 36 barley cultivars against Cochliobolus sativus leaf spot disease (continued)

Barley Cultivars	Replications	Mean	Infection Classe
÷	5		
Sinanbey	5	5	В
	5		
	7		
Tarm 92	7	7	С
	7		
	6		
Tosunpaşa	6	6	С
-	6		
	7		
Yalın	6	6.33	С
	6		
	5		
Yaprak	5	4.67	В
	4		
	5		
Yesevi 93	5	5.33	В
	6		
	7		
Yüksel	6	6.33	С
	6		
	6		
Zeynelağa	6	5.67	С
	5		
	5.75		
General Mean	5.56	5.65	С
	5.64		

 Table 2 (Continued). Seedling stage reactions of 36 barley cultivars against Cochliobolus sativus leaf spot disease (continued)

(1-9 scale, Fetch and Steffenson, 1999; scale values; a: 1-3 low, b: 4-5 intermediate, c: 6-9 high)

 Table 3. Seedling stage reactions of 21 wild barley (Hordeum spontaneum) genotypes against Cochliobolus sativus leaf spot

 disease

Hordeum spontaneum genotypes	Replications	Mean	Infection Classes
	4		
TRNC 1	5	4.67	В
	5		
	7		
TRNC 2	7	7	С
	7		
	7		
TRNC 3	7	7	С
	7		
	5		
TRNC 4	5	5	В
	5		

Hordeum spontaneum genotypes	Replications	Mean	Infection Classes
	5		
TRNC 5	5	5	В
	5		
	6		
1	7	6.67	С
	7		
	6		
2	6	5.67	С
	5		
	4		
3	5	4.67	В
	5		
	5		
9	5	5	В
	5		
	6		
21	6	6	С
	6		
	6		
22	5	5.33	В
	5		
	3		
23	3	3.33	А
	4		
	7		
24	7	6.67	С
	6		
	3		
26	4	3.67	В
	4		
	7		
27	6	6.33	С
	6		
	7		
28	6	6.33	С
	6		
	7		
29	7	6.67	С
	6		_
	2		
30	2	2	А
	2	-	11

 Table 3 (Continued). Seedling stage reactions of 21 wild barley (Hordeum spontaneum) genotypes against Cochliobolus sativus leaf spot disease (continued)

Hordeum spontaneum genotypes	Replications	Mean	Infection Classes
	6		
31	6	5.67	С
	5		
	5		
32	5	5	В
	5		
	6		
33	6	6.33	С
	7		
	5.43		
General Mean	5.48	5.43	В
	5.38		

 Table 3 (Continued). Seedling stage reactions of 21 wild barley (Hordeum spontaneum) genotypes against Cochliobolus sativus leaf spot disease (continued)

4. Discussion

In different studies, the resistance status of barley genotypes and the virulence status of the isolates used were investigated. Aktaş and Tunalı (1994) investigated the leaf spot resistance status of barley genotypes against the S96 strain of *C. sativus*. The barley cultivars and lines used in this study showed very susceptible, susceptible, moderately susceptible, and moderately resistant reactions.

Jana and Bailey (1995) evaluated the responses of wild and cultivated barleys obtained from Türkiye and Jordan against three leaf pathogens, including C. sativus. They found that 4.5% of wild barleys and 0.3% of cultured barleys were resistant to C. sativus. Fetch et al. (2003) determined the reactions of a total of 116 H. spontaneum genotypes obtained from Israel and Jordan against 6 fungal pathogens, including leaf spot disease caused by C. sativus. As a result of their study, they found the resistance frequency against Cochliobolus leaf spot as 53% and 46% from Israel and Jordan genotypes. In our study, 5.56% of 36 barley cultivars, 11.43% of 35 barley lines, and 9.52% of 21 wild barley genotypes were found to be resistant (showing low infection response). Arabi and Jawhar (2004) investigated the infection responses of 10 barley genotypes against 12 C. sativus isolates obtained from different regions of Syria and determined that the reactions of the genotypes ranged from susceptible to moderately resistant. In this study, no genotype was immune to the disease Bonman et al. (2005) evaluated the resistance of barley genotypes obtained from the National Small Grains Collection (USA) against Cochliobolus leaf spot disease and found 3 of 48 genotypes to be resistant. Ghazvini and Tekauz (2007) evaluated 160 barley cultivars from Iran in order to determine their responses to head blight, leaf spot, and net blotch diseases. As a result of the study, no resistant barley cultivars were found against leaf spot and head blight. In our study, we found resistant cultivars, lines, and wild barley genotypes using a virulent strain of C. sativus.

Çelik Oğuz et al. (2016) determined the seedling reactions of 25 barley lines against 5 single spore isolates of *C. sativus*. Statistically significant differences (P<0.01) were observed among the isolates, and no barley line showed a low infection response. Two barley genotypes exhibited a moderate infection response to all 5 isolates. Six genotypes used in this current study showed low infection response against *C. sativus* isolate. Singh et al. (2017), under natural conditions, determined the responses of 342 barley genotypes to *Cochliobolus* leaf spot disease in their study and found 97 genotypes to be moderately resistant and one

genotype to be resistant. In our study, 8 of 92 barley cultivars, lines, and barley genotypes were resistant and 37 of them gave intermediate reactions.

Celik-Oguz and Karakaya (2017), under greenhouse conditions, determined the seedling stage responses of 39 barley cultivars widely grown in Türkiye against three isolates of *C. sativus*. As a result of the research, differences were observed in the responses of different barley cultivars to pathogen isolates, and Cs1 was the most virulent isolate. Avci 2002 cultivar showed moderate infection response to Cs3 isolate and high infection response to Cs1 and Cs2isolates. Akar, Aydanhanim, Bolayir, Burakbey, Bülbül 89, Çetin 2000, İnce 04, Orza 96, Özdemir 05, Tarm 92, Yesevi 93, and Zeynelağa cultivars showed high infection response to all three isolates. In this current study, cultivars Akar, Bolayir, Orza 96, and Yesevi 93 showed moderate infection response against *C. sativus* isolatewhile Avci 2002, Aydanhanim, Burakbey, Bülbül 89, Çetin 2000, İnce 04, Özdemir 05, Tarm 92 and Zeynelağa cultivars showed high infection response.

Balcı et al. (2018) evaluated the seedling stage infection responses of one hulless barley cultivar candidate, 2 hulless barley cultivars, and 19 hulless barley genotypes against two isolates of *C. sativus* under greenhouse conditions. Differences in virulence were detected between the isolates. Hulless barley cultivars Yalın and Özen showed moderate infection response to both isolates. A high infection response of cultivar Yalın was observed against *C. sativus* isolate used in this current study.

Çelik Oğuz et al. (2019) determined the seedling stage reactions of 28 six-row barley landraces, as well as Avcı 2002 and Bülbül 89 cultivars, against two *C. sativus* isolates. Avcı 2002 cultivar showed moderate infection response against Kastamonu isolate and low infection response against Hatay isolate; Bülbül 89 cultivar exhibited high and moderate infection responses against Kastamonu and Hatay isolates, respectively. In this current study, Avcı 2002 and Bülbül 89 cultivars showed a high infection response against the *C. sativus* isolate used.

5. Conclusions

Barley, which is one of the main field crops in Türkiye and the World, has an important place in terms of cultivation area and production amount. Breeding studies are important in order to obtain new cultivarsthat can tolerate diseases and pests well and have high adaptability.

Cochliobolus leaf spot disease in barley is an important disease that reduces yield and quality. In this study, barley lines and varieties and wild barley (*Hordeum spontaneum*) genotypes resistant to *Cochliobolus* leaf spot disease caused by *C. sativus* were determined. These resistant barley lines and varieties and wild barley genotypes are among the gene sources that can be used in breeding work. Further studies should be conducted on the resistance of barley against diseases and breeding studies should be carried out in order to develop disease-resistant varieties.

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