

## Determination of the seedling reactions of six-rowed barley landraces to spot blotch disease incited by *Cochliobolus sativus*

### *Altı sıralı arpa köy çeşitlerinin Cochliobolus sativus tarafından oluşturulan yaprak lekesi hastalığına karşı fide dönemi tepkilerinin belirlenmesi*

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#### ABSTRACT

Spot blotch disease incited by *Cochliobolus sativus* is an important disease of barley (*Hordeum vulgare* L.) crops worldwide. In this study, seedling reactions of 28 six-rowed barley landraces obtained from Osman Tosun Gene Bank, Turkey, and two Turkish barley cultivars (Avcı 2002 and Bülbül 89) to 2 *Cochliobolus sativus* isolates obtained from Kastamonu and Hatay provinces of Turkey were determined. Virulence difference between the isolates was observed. Isolate obtained from Kastamonu province was more virulent. Two, 18, and 8 landraces showed low, intermediate, and high infection responses to Kastamonu isolate, respectively. Cultivar Avcı 2002 showed intermediate infection response and cultivar Bülbül 89 showed high infection response to Kastamonu isolate. Nine, 18, and 1 landraces showed low, intermediate and high infection responses to Hatay isolate, respectively. Cultivar Avcı 2002 showed low infection response and cultivar Bülbül 89 showed intermediate infection response to Hatay isolate. Landraces 12 and 24 showed low infection responses to both isolates. Landraces 6, 8, 13, 16, 17, and 27 showed low and intermediate infection responses to Hatay and Kastamonu isolates, respectively. Landraces 3, 4, 5, 9, 10, 11, 18, 21, 23, 26, and 28 showed intermediate infection responses to both isolates. Barley landraces showing low and/or intermediate infection responses to *Cochliobolus sativus* could be used in spot blotch resistance breeding studies or can be planted in areas where spot blotch is common.

**Key Words:** Spot blotch, *Cochliobolus sativus*, *Bipolaris sorokiniana*, Barley landraces, Disease resistance

#### ÖZ

*Cochliobolus sativus* fungusunun neden olduğu *Cochliobolus* yaprak lekesi arpa (*Hordeum vulgare* L.) bitkisinin dünya çapında önemli bir hastalığıdır. Bu çalışmada Osman Tosun Gen Bankası'ndan elde edilen 28 adet altı sıralı arpa köy çeşidi ve 2 adet Türk arpa çeşidinin (Avcı 2002 ve Bülbül 89), Türkiye'nin Hatay ve Kastamonu illerinden elde edilen 2 adet *Cochliobolus sativus* izolatına karşı fide dönemi tepkileri belirlenmiştir. İzolatlar arasında virülens farklılığı gözlenmiştir. Kastamonu ilinden elde edilen-izolat daha virulent olarak bulunmuştur. İki, 18 ve 8 adet arpa köy çeşidi Kastamonu izolatına sırasıyla düşük, orta ve yüksek enfeksiyon tepkileri göstermiştir. Avcı 2002 çeşidi Kastamonu izolatına orta enfeksiyon tepkisi verirken Bülbül 89 çeşidi yüksek enfeksiyon tepkisi vermiştir. Dokuz, 18 ve 1 arpa köy çeşidi Hatay izolatına sırasıyla düşük, orta ve yüksek enfeksiyon tepkileri vermiştir. Avcı 2002 çeşidi Hatay izolatına düşük enfeksiyon tepkisi gösterirken Bülbül 89 çeşidi orta enfeksiyon tepkisi vermiştir. On iki ve 24 numaralı arpa köy çeşitleri her iki izolata da düşük enfeksiyon tepkisi göstermişlerdir. Altı, 8, 13, 16, 17 ve 27 numaralı arpa köy çeşitleri Hatay ve Kastamonu izolatlarına sırasıyla düşük ve orta enfeksiyon tepkileri göstermişlerdir. Üç, 4, 5, 9, 10, 11, 18, 21, 23, 26 ve 28 numaralı arpa köy çeşitleri ise her iki izolata da orta enfeksiyon tepkisi göstermişlerdir. *Cochliobolus sativus*'a karşı düşük veya orta enfeksiyon tepkileri gösteren arpa köy çeşitleri *Cochliobolus* yaprak lekesi hastalığına dayanıklılık çalışmalarında kullanılabilir veya hastalığın yaygın olduğu alanlarda ekilebilir.

**Anahtar Kelimeler:** Yaprak lekesi, *Cochliobolus sativus*, *Bipolaris sorokiniana*, Arpa köy çeşitleri, Hastalıklara dayanıklılık

## Introduction

Barley (*Hordeum vulgare* L.) is the second most produced cereal crop after wheat in Turkey. The origin of *Hordeum vulgare* is thought to be the Fertile Crescent region which is located in the Tigris and Euphrates valleys along with the Mediterranean to Arabian Gulf regions (Harlan, 1995; Nesbitt, 1995; Willcox, 1995; Ladizinski, 1998). Landraces are heterogeneous population of plant varieties produced by farmers under both artificial and natural selection processes (Brown, 2000). Barley landraces are important genitors that are still being cultivated. They are important as a germplasm source for barley breeding studies and for enhancing the genetic diversity of barley. Barley landraces have a wide adaptation range to biotic and abiotic 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 (Ceccarelli et al., 2000; Ceccarelli and Grandi, 2000).

Barley landraces emerged as a result of many years of selection and they are well adapted to the climate and soil conditions of the relevant region. Barley landraces endured adverse conditions for a long number of years and gained resistance to the local insects and diseases. However, with the emergence of high-yielding and high-quality commercial varieties, farmers used commercial varieties more and abandoned their local varieties. Over time, loss of landraces with a high degree of variation caused genetic erosion, and narrowed the variation. The risk of disappearance of landraces, an important gene source, is present. Therefore, collection and storage studies of landraces have been started. For this purpose, genetic stock studies were initiated in 1938 by Osman Tosun at the Institute of Agronomy and Plant Breeding, Ankara Higher Agriculture College for the first time in Turkey. Barley landraces have been collected from different parts of Turkey, where one of the centers of the origin of the cultivated barleys, by Osman Tosun and his colleagues. Barley germplasm from several countries are also maintained in Osman Tosun Gene Bank, Ankara, Turkey.

*Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker) is an important pathogen of barley (Sivanesan, 1987). The fungus is the causal agent

of common root rot and spot blotch, and limits the barley yield (Mathre, 1982). Spot blotch generally occurs in moist environmental conditions. Disease symptoms are chlorotic and necrotic areas on the barley leaf (Fetch and Steffenson, 1999). Depending on the environmental conditions, yield losses due to disease range from 16 to 33 % (Wilcoxson et al., 1990).

Although fungicides could be used to control the spot blotch pathogen, the most environmentally friendly and effective control method is the use of resistant barley genotypes (Kiesling, 1985). In this study, 28 six-rowed barley landraces and two Turkish cultivars (Avcı 2002 and Bülbül 89) were tested using 2 *Cochliobolus sativus* isolates for resistance to spot blotch disease. An abstract of this study has been published previously (Çelik Oğuz et al., 2018).

## Materials and Methods

### *Plant materials and the pathogen*

Twenty-eight six-rowed barley landraces obtained from Osman Tosun Gene Bank and two Turkish barley cultivars (Avcı 2002 and Bülbül 89) were used as plant materials. Six-rowed landrace seeds were multiplied from single spikes. Two single spore isolates of *Cochliobolus sativus* obtained from Hatay and Kastamonu provinces of Turkey were used as pathogen isolates.

### *Inoculation and disease assessment*

The inoculum was prepared from 12 days old *Cochliobolus sativus* isolates grown in Potato Dextrose Agar medium and adjusted to  $2 \times 10^4$  conidia / ml. Ten seeds of each landrace and two Turkish cultivars Avcı 2002 and Bülbül 89 were planted in 7 x 7 cm pots. Inoculum was sprayed onto the plants with a hand spray machine and plants were kept in the nylon covered boxes at  $15-22 \pm 3$  °C for night / day with a 12h / 12h light / dark regime in a greenhouse for 3 days. There were three replications. The plants were evaluated 10 days after inoculation with a scale, divided into 3 different infection response (IR) categories (low, intermediate, and high), developed by Fetch and Steffenson (1999).

### *Data analysis*

Analysis of variance was performed for the

determination of virulence difference between the isolates. Biplot graphics based on PCA analysis was accomplished by using 2 main components (isolates x scale values) to evaluate the response of genotypes to isolates (MSTAT, Michigan State University, East Lansing, Michigan, USA).

## Results and Discussion

Kernel colors of 28 six-rowed barley landraces and seedling reactions of 28 six-rowed barley landraces and 2 Turkish barley cultivars to 2 isolates of *Cochliobolus sativus* were presented in Table 1. Variation in the reactions of barley landraces to *Cochliobolus sativus* isolates was observed. Kastamonu isolate was found to be

more virulent than the Hatay isolate. Landraces 3, 4, 5, 9, 10, 11, 18, 21, 23, 26, and 28 showed intermediate infection responses to both isolates. Landraces 6, 8, 13, 16, 17, and 27 showed low infection responses to Hatay isolate and intermediate infection responses to Kastamonu isolate. Landraces 12 and 24 showed low infection responses to both isolates. Cultivar Avcı 2002 showed intermediate infection response and low infection response to Kastamonu and Hatay isolates, respectively. Cultivar Bülbül 89 showed high infection response and intermediate infection response to Kastamonu and Hatay isolates, respectively (Table 1).

Table 1. Kernel colors of 28 six-rowed barley landraces and seedling reactions of 28 six-rowed barley landraces and 2 Turkish barley cultivars to 2 isolates of *Cochliobolus sativus* obtained from Kastamonu and Hatay provinces of Turkey. For assessment of the disease a scale developed by Fetch and Steffenson (1999) was used. In this scale 3 categories of infection responses (IR) were recognized (low (IR 1-3), intermediate (IR 4-5) and high (IR 6-9)).

Çizelge 1. Yirmi sekiz adet altı sıralı arpa köy çeşidinin tane renkleri ve 28 altı sıralı arpa köy çeşidi ve 2 Türk arpa çeşidinin, Türkiye'nin Kastamonu ve Hatay illerinden elde edilen *Cochliobolus sativus*'un 2 izolatına fide dönemi tepkileri. Hastalık değerlendirmesinde Fetch ve Steffenson (1999)'ın geliştirdiği iskala kullanılmıştır. Bu iskalada 3 tip enfeksiyon tepkisi (IR) düşük (IR 1-3), orta (IR 4-5) ve yüksek (IR 6-9) tanımlanmıştır.

Landrace No/ Osman Tosun Gene Bank accession number Köy çeşidi No/ Osman Tosun Gen Bankası erişim numarası	Kernel color Tane rengi	Infection response Enfeksiyon tepkisi		
		Kastamonu isolate Kastamonu izolatu	Hatay isolate Hatay izolatu	
1	246	White	6	4
2	205	White	7	4
3	305	White	5	4
4	309	White	5	4
5	102	Greyish	5	4
6	56	White	4	3
7	122	White	7	4
8	296	White	5	3
9	174	White	5	4
10	62	White	5	4
11	141	White	4	4
12	80	White	3	3
13	81	White	5	3
14	337	White	6	4
15	7	White	6	3
16	301	White	4	3
17	135	White	4	3
18	326	White	5	5
19	297	White	5	6
20	202	White	6	5
21	340	White	5	4
22	194	White	7	4
23	281	Greyish	4	4
24	119	Black	3	3
25	61	White	6	4
26	96	White	5	4
27	157	White	4	3
28	14	White	5	4
Bülbül 89		White	8	5
Avcı 2002		White	5	3
Mean			<b>5,13 A*</b>	<b>3,83 B*</b>

\*Significant at P<0.001 probability level (F Ratio=26,43)

Virulence level of the isolates varied. Difference between the isolates was statistically significant ( $P < 0.001$ ). Biplot analysis revealed that genotypes with the lowest infection responses were located at the left of the median line. Genotypes 24 and 12 which were resistant (LIR) to the both isolates were grouped at the same point. They were placed at the lower left corner of graphics farthest from the isolate lines. Genotypes 11 and 23 which received scale values 4 were located in close proximity to the median line. Genotypes 27, 17, 6, and 16 received 4 and 3 scale values to Kastamonu and Hatay isolates, respectively. These isolates were more susceptible to Kastamonu isolate, therefore, they gathered at the same point in the upper left

corner of the graphics close to Kastamonu isolate line. Genotypes 13, 8, and barley cultivar Avci 2002 received scale values of 5 and 3 to Kastamonu and Hatay isolates, respectively. All of these 3 genotypes showed resistant reaction to the Hatay isolate, and they were placed at the opposite direction of Hatay isolate in the graphics. Similar response was observed in genotype 15 which was resistant to Hatay isolate. Genotypes located at the right side of graphics showed intermediate or high infection responses. Barley cultivar Bülbül 89 showed high- and intermediate infection responses to Kastamonu and Hatay isolates, respectively. This cultivar was placed in between 2 isolates with a leaning towards to the Kastamonu isolate line (Figure 1).

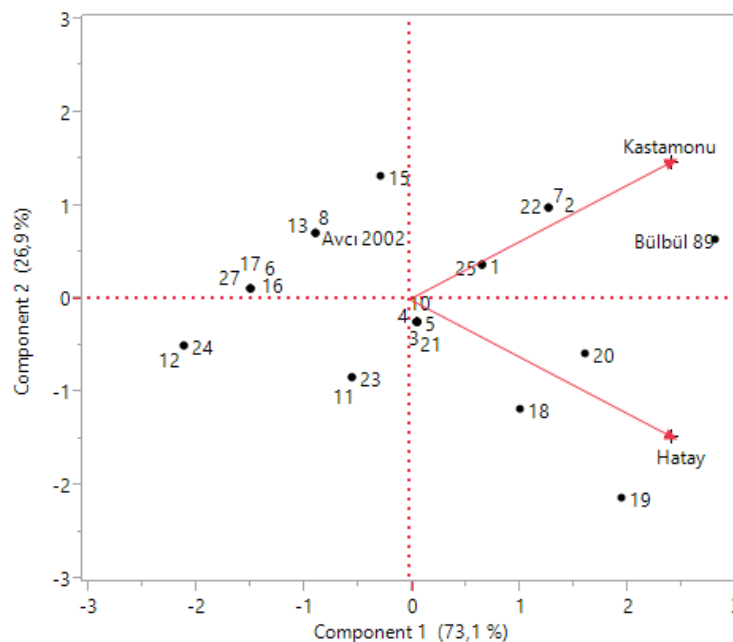


Figure 1. Biplot graphics based on PCA analysis of infection responses on six-rowed barley landraces.

Çizelge 1. Biplot grafiği, altı sıralı yerel arpa genotiplerinin enfeksiyon tepkisinin PCA analizi temel alınarak oluşturulmuştur.

There are limited number of studies related to the spot blotch disease and resistance to this disease. Aktaş and Tunalı (1994) investigated the resistance of some barley cultivars and genotypes to *Cochliobolus sativus* race S96. The barley line KABVD-2017 showed moderate resistant reaction. In a study carried out by Çelik Oğuz et al. (2016) seedling stage responses of 25 advanced barley lines to 5 singlespore isolates of *Cochliobolus sativus* were determined under greenhouse conditions. The most virulent isolate was found as Yozgat isolate. Two barley genotypes exhibited a

moderate infection response to all 5 isolates. In our current study, eleven (39.2 %) landraces showed intermediate reaction to both isolates. Balcı et al (2018) determined seedling reactions of some hulless barley cultivars and barley genotypes to *Cochliobolus sativus*. Virulence difference between the isolates was observed. Yozgat isolate was found as more virulent. Hulless barley cultivars Yalın and Özen showed intermediate infection responses. The reactions of hulless barley genotypes ranged between intermediate- and high infection response. A

hulless cultivar candidate showed low infection response. Celik-Oguz and Karakaya (2017) determined seedling response of 39 cultivars of barley commonly grown in Turkey to spot blotch disease agent under greenhouse conditions. The reactions of the cultivars of barley differed in their response to isolates. In their study, differences in virulence levels among the isolates were evident. Isolate Cs1 was the most virulent isolate. Cultivar Vamıkhoca 98 was found to be the most resistant one among the cultivars tested. Cultivar Avcı 2002 showed high infection response to 2 isolates and intermediate infection response to 1 isolate. Cultivar Bülbül 89 exhibited high infection response to all 3 isolates. In our current study, landraces 12 and 24 showed low infection responses to both isolates and all tested barley landraces showed intermediate- or low infection responses to at least 1 isolate. Cultivar Avcı 2002 showed intermediate infection response and cultivar Bülbül 89 showed high infection response to Kastamonu isolate. Barley cultivar Avcı 2002 showed low infection response and cultivar Bülbül 89 showed intermediate infection response to Hatay isolate. Bonman et al. (2005) found that 3 of the 48 barley accessions obtained from USDA National Small Grains Collection were resistant to spot blotch disease. In another study, Singh et al. (2017) evaluated 342 barley genotypes against spot blotch disease under natural conditions, Only 1 genotype was found to be resistant and 97 genotypes showed moderately resistant reactions. In our current study, 2 out of 28 six-rowed barley landraces exhibited low infection responses to both isolates.

Ethiopia (Alemayehu and Parlevliet, 1997), Iran (Brown and Munday, 1982), Jordan (Jaradat et al., 1987), Sardinia (Papa et al., 1998), Syria (Ceccarelli et al., 1987) and Turkey (Brush, 1995; Kün, 1996) are the main gene centers of barley. Landraces from these areas have high potential for improved agronomical traits as well as disease resistance. Legge et al. (1990) evaluated 176 Turkish barley accessions for their reactions to barley pathogens prevalent in Canada. They concluded that this germplasm was not a good

source of resistance to *Cochliobolus sativus* (spot blotch phase). Jana and Bailey (1995) evaluated wild and cultivated barley landraces (*Hordeum vulgare* subsp. *spontaneum* and *H. vulgare* subsp. *vulgare*) obtained from Turkey and Jordan to three important foliar pathogens (*Pyrenophora teres* f. *teres*, *Pyrenophora teres* f. *maculata* and *Cochliobolus sativus*). 4.5 % of wild barley accessions and 0.3 % of cultivated barley accessions were found to be resistant to *Cochliobolus sativus*. Arabi and Jawhar (2004) tested 10 barley genotypes collected from different regions of Syria against 12 isolates of *Cochliobolus sativus*. The reactions of barley genotypes ranged between very susceptible to moderately resistant. Isolate C41 showed differential virulence pattern. Ghazvini and Tekauz (2007) evaluated the reactions of 160 barley accessions from Iran to head blight, spot blotch, and net blotch diseases of barley. No barley accession was found to be resistant to spot blotch and head blight diseases. In our current study, 32.14 % of six-rowed barley landraces showed resistant reactions to at least one isolate.

Steffenson et al., (1996) and Wilcoxson et al., (1990) emphasized the use of disease resistant six-rowed malt barley varieties. These varieties reduced the prevalence of spot blotch disease in the last 30 years in the midwest region of the USA. In our current study, six-rowed barley landraces that showed low- or intermediate infection responses could be used as a source of resistance to spot blotch disease.

Narrowed and uniformed barley gene pools are inadequate for the development of new barley varieties with improved tolerance to biotic and abiotic stress factors. One of the primary gene sources which can be used in the breeding programs to develop new varieties with greater adaptation abilities that will better tolerate the changing climate and soil conditions as well as insect and disease factors is the landraces. New varieties with improved yields and higher quality have been generated using elite materials (modern varieties) in the breeding programs. On the other hand, the use of elite (modern)

materials in plant breeding programs have led to the genetic uniformity of varieties, resulting in both narrowing of the variation and greater vulnerability to biotic and abiotic stress factors caused by climate change (Munoz-Amatriain et al., 2014a; Munoz-Amatriain et al., 2014b). The barley genetic resource pool needs to be expanded to develop improved varieties that have resistance to biotic and abiotic stress factors.

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