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Determination of the reactions of some barley (*Hordeum vulgare* L.) landraces and cultivars to *Drechslera graminea*

Bazı arpa (*Hordeum vulgare* L.) köy çeşitleri ve arpa çeşitlerinin *Drechslera graminea'* ya tepkilerinin belirlenmesi

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ABSTRACT

Reactions of 20 landraces and three cultivars of barley (*Hordeum vulgare* L.) to leaf stripe disease were evaluated under greenhouse conditions. Ten *Drechslera graminea* isolates collected in ten locations of Turkey were used for inoculation using the sandwich method. Phenotypic variation to leaf stripe disease was observed in the responses of landraces and cultivars of barley with the same and different isolates of the fungus. Barley landraces #3 and #5 exhibited resistance and susceptibility to eight isolates of the fungus, respectively. Barley cultivar Çumra 2001 showed a resistant reaction to all isolates. Cultivars Atılır and Larende were susceptible to 9 isolates. Virulence differences were observed among the fungal isolates. The *D. graminea* Konya (Bozkır) isolate was the most virulent while Ankara (Haymana) isolate was the least virulent. This research shows that barley landraces and cultivars could be a rich source of phenotypic variability against current strains of *D. graminea* found in barley production areas of Turkey. Resistant cultivar and landraces could be used in breeding programs.

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ÖZ

Yirmi arpa (*Hordeum vulgare* L.) köy çeşidinin ve 3 arpa çeşidinin arpa çizgili yaprak lekesi hastalığına karşı tepkileri sera şartlarında belirlenmiştir. Türkiye'nin 10 değişik bölgesinden elde edilen *Drechslera graminea* izolatları sandviç yöntemi ile yapılan inokulasyonda kullanılmışlardır. Arpa köy çeşitleri ve arpa çeşitlerinin fungusun aynı ve değişik izolatlarına karşı tepkilerinde fenotipik varyasyon görülmüştür. Fungusun 8 izolatına karşı arpa köy çeşitlerinden 3 numaralı çeşit dayanıklı reaksiyon verirken 5 numaralı çeşit hassas reaksiyon vermiştir. Çumra 2001 arpa çeşidi bütün izolatlara karşı dayanıklı reaksiyon vermiştir. Atılır ve Larende arpa çeşitleri 9 izolata karşı hassas reaksiyon vermişlerdir. Fungus izolatları arasında virülenslik bakımından farklılıklar görülmüştür. *D. graminea* Konya (Bozkır) izolatı en virulent izolat olarak, Ankara (Haymana) izolatı ise virülensi en düşük izolat olarak bulunmuştur. Bu araştırma arpa köy çeşitleri ve arpa çeşitlerinin Türkiye'de arpa üretim alanlarında görülen *D. graminea* 'nın streynlerine karşı zengin bir fenotipik varyasyon kaynağı olabileceğini göstermiştir. Dayanıklı çeşit ve köy çeşitleri ıslah programlarında kullanılabilir.

1. Introduction

Barley (*Hordeum vulgare* L.) is an important crop both in the world and in Turkey. It is thought that barley was first cultivated in Fertile Crescent. Barley is used as an animal feed and in malt industry (von Bothmer and Jacobsen 1985, Geçit et al. 2009). Barley is one of the oldest plants cultured and barleys have been grown in Turkey for a very long time. Turkey is one of the important gene centers for barley (Kün 1996). Many barley landraces are grown throughout Turkey.

Barley is grown in the world in 48 million ha area with a production of 124 million tonnes. The world mean for barley yield is 2596 kg/ha. In Turkey, barley is grown in 3 million ha area with a production of 7,3 million tonnes. The mean yield of barley in Turkey is 2451 kg/ha (Anonymous 2010).

Because of their large genotypic variation, barley landraces have better adaptation to biotic and abiotic stress factors and adverse environmental conditions compared to commercial cultivars. Barley landraces have genes for tolerance to abiotic stresses such as drought, cold and salt stress as well as resistance genes to various pathogens. These genes could be used in breeding programs (Allard and Bradshaw 1964).

Anatolian barley landraces were found to be superior to other barley cultivars regarding protein content, 1000 kernel weight and yield. In Russia, Anatolian barley landraces collected by Zhukowsky were planted between 1925-1927. Among these 11 different varieties were recognized. A total of 1122 samples were planted. 1000 kernel weights, hectoliter weights and protein contents of these samples varied between 30-62 g, 62.0-70.9 g and 8-12% respectively. The yield of Anatolian barley landraces were higher than standard Russian cultivars and cultivars obtained from other countries. Also in the same study, it was found that hulless Anatolian barleys were better in yield and drought resistance (Gökgöl 1969).

In another study, 44 two rowed and 52 six rowed barley lines obtained from Ankara University Osman Tosun Gen Bank were planted under Ankara, Turkey conditions. In this study, significant differences among the traits like days to heading, plant height, number of kernels per head, 1000 kernel weight, kernel yield in spikes, and yield were observed (Çakır 1988).

In a study carried out by ICARDA, 19652 barley lines and cultivars obtained from 61 countries were evaluated. Seven percent of these were barley landraces obtained from Turkey. Turkish materials proved to contain variation for traits important in barley breeding. For example, the highest 1000 kernel weights (up to 78 g) identified in the study were in two and six rowed Turkish landraces (ICARDA 1998).

Barley stripe disease caused by the fungus *Pyrenophora graminea* (S. Ito & Kurib.) is decreasing the barley yield worldwide. Anamorphic stage of the fungus is named *Drechslera graminea* (Rabenh. ex Schlecht.) Shoemaker (=*Helminthosporium gramineum* Rabh.). Disease symptoms can be seen from tillering stage to maturity. Initial symptoms start as yellow stripes in seedling leaves. Later on these stripes turn to brown and necrotic areas develop that can tear the leaf blade horizontally. Spikes can fail to develop. When the disease is severe complete drying of the plant is also observed (Mathre 1982).

In Turkey, this disease is present in winter barley growing areas (Mamluk et al. 1997). Karakaya et al. (2014) found the disease in 40% of the fields inspected in the Central Anatolian region of Turkey. The disease can cause yield losses up to 10-15% (Aktaş 2001). In the Turkish province of Şanlıurfa, disease severity during the tillering stage was 3.2% and increased to 5.3% at crop maturity. Symptoms observed included plants without spikes (2.8%), absence of kernels (1.5%) and kernel malformation (1.0%). Yield losses due to disease were estimated as 5% in 2002. It is concluded that in addition to yield loss, quarantine aspect of the disease was also important (Kavak 2004).

Genetic resistance to barley leaf stripe is an effective and economically sound way of lowering the disease incidence in Turkish barley crops. Modern barley cultivars showed genetic variation to the disease. It was demonstrated that barley cultivars Durusu, Balkan 96 (Igri), Çumra 2001 and Anadolu 98 were resistant (Ulus and Karakaya 2007; Bayraktar and Akan 2012). In another study done in Canada, Tekauz (1983) showed that out of 57 barley cultivars 9 cultivars showed a resistant reaction.

Because barley is an important crop for the agriculture of Turkey, there is a need for searching additional new sources of resistance to leaf stripe disease, and for that Turkish landraces and current cultivars need to be evaluated and compared. Additionally, it has been demonstrated that *Pyrenophora graminea* populations showed diversity in virulence toward barley and in morphological characters (Hammouda 1988; Gatti et al. 1992; Arabi and Jawhar 2004; Jawhar and Arabi 2006). Pathogen populations that are genetically diverse can more rapidly evolve and overcome crop genetic resistance compared to less diverse populations (McDonald and Linde 2002). Understanding genetic variability of both plant and fungus may lead to develop better control strategies.

In this study, we evaluated the leaf stripe response of barley landraces and cultivars to a set of ten isolates of *D. graminea* collected in ten locations of Turkey. An abstract of this study has been published (Çelik et al. 2014).

2. Materials and Methods

This study was carried out at the laboratory and greenhouse of Central Research Institute for Field Crops located in Ankara, Turkey. Diseased barley samples were obtained from ten different regions of Turkey (Konya-Altınekin, Konya-Bozkır, Ankara-Akyurt, Ankara-Haymana, Eskişehir-Sivrihisar, Bilecik, Afyon-Çay , Kayseri, Sivas and Yozgat) during May and June of 2012. One isolate was obtained from each location. Growth and colony characteristics of isolates were examined after 10 days of growth in PDA.

Seeds of 20 barley landraces and 3 barley cultivars were obtained from Central Research Institute for Field Crops, Ankara, Turkey. Some information about the landraces and cultivars are given in Table 1.

Table 1. Some information about the landraces and cultivars used in this study.

Landrace/Cultivar	Places of landraces obtained	Type	Kernel color
1	Ankara	2 row	white
2	Ankara-Ag. Res. Inst.	2 row	white
3	Ankara- Ag. Res. Inst.	2 row	white
4	Ankara- Ag. Res. Inst.3042	2 row	white
5	Ankara- Ag. Res. Inst.3174	6 row	white
6	Ankara- Ag. Res. Inst.3410	2 row	white
7	Uşak-Central District	2 row	white
8	Uşak-Eşme	2 row	white
9	Afyon-Bolvadin	2 row	white
10	Afyon-Sandıklı	6 row	white
11	Afyon-Emirdağ	2 row	white
12	Afyon-Emirdağ	2 row	black
13	Afyon-Emirdağ	2 row	black
14	Uşak-Sivaslı	2 row	white
15	Afyon-Central District	2 row	white
16	Ankara187	2 row	white
17	Ankara470	6 row	black
18	Ankara529	6 row	white
19	Ankara541	2 row	white
20	Ankara977	2 row	black
Atılır	-	2 row	white
Larende	-	2 row	white
Çumra2001	-	2 row	white

To isolate the pathogen isolates, small leaf segments were surface sterilized for 1 min with 1% NaOCl and placed into Petri plates containing Water Agar (WA). The plates were incubated 3-4 days at room temperature. After 3-4 days hyphal tips were cut under a dissecting microscope and transferred to Petri plates containing Potato Dextrose Agar (PDA). They were later moved to PDA slants and kept at 4 ºC.

For the barley stripe disease phenotype tests, the barley seed sandwich method described by Mohammad and Mahmood (1974) were used. Three replicate plates were used for each isolate. Barley seeds were surface sterilized 3 minutes using 1% NaOCI solution and then rinsed with sterile distilled water. Under aseptic conditions, fifteen seeds were placed onto half of 90 mm Petri plates containing 10-day-old cultures of *D. graminea* in Potato Dextrose Agar. Fungal cultures were maintained at 22°C. The other half of the agar was folded on the seeds and a sandwich was formed. These plates were maintained 72 hours at 22°C under light. Depending on the germination status of seeds they were further incubated 5-7 days at 4 °C.

Incubated seeds were taken from the sandwiches carefully using sterile forceps and placed into pots, 16 cm in diameter, containing sand, animal manure, and soil (w:w 1:1:3). Fifteen seeds were placed in each pot. There were 3 replications. Plants were grown in a greenhouse with a night/day temperature of 10─22 ± 3 °C.

Disease evaluations were performed 60 days after planting the germinated seeds to the pots using a scale developed by Tekauz (1983). In this scale values were:

1: Resistant (infection % < 5%) = R

2: Intermediate (infection 5-17%) = I

3: Susceptible (infection% > 17%) = S

3. Results and Discussion

Seedling reactions of 3 barley cultivars and 20 barley landraces were determined using 10 different *D. graminea* isolates collected from various regions of Turkey. Emergence of the plants started 3 days after planting the germinated seeds to pots. Disease symptoms started in second week. At the end of 6th week symptoms were evident in other susceptible cultivars. Differences were observed among the reactions of the cultivars to the isolates of the fungus. There were also pathogenicity differences among the isolates (Tables 2 and 3). All reaction types were present.

Çumra 2001 cultivar exhibited a resistant reaction to all isolates. Cultivars Atılır and Larende showed a susceptible reaction to 9 isolates and an intermediate reaction to 1 isolate. Barley landrace 3 showed a resistant reaction to 8 isolates and an intermediate reaction to 2 isolates. No susceptible reaction was observed with this landrace to isolates. Barley landrace 8 showed a resistant reaction to 5 isolates and landraces 2, 17 and 19 were resistant to 4 isolates. These landraces were the most resistant landraces used in this study. Barley landrace 13 showed a susceptible reaction to 9 isolates. Barley landraces 5 and 10 showed a susceptible reaction to 8 isolates. These landraces were the most susceptible landraces used in this study. Although differences in resistance to the isolates were observed, the majority of the landraces showed susceptible reactions to most of the isolates.

Table 2. Determination of seedling reactions of 3 barley cultivars and 20 landraces to 10 *Drechslera graminea* isolates. For evaluation, a 1-3 scale developed by Tekauz (1983) was used. (R: Resistant, MR: Moderately Resistant, S: Susceptible). Numbers are mean of three replications.

Barley landraces and cultivars	Isolates										Mean
	Konya (Altınekin)		Konya (Bozkır)		Ankara (Akyurt)		Ankara (Haymana)		Eskişehir		
	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value	
1	28.10	3 (S)	23.49	3 (S)	4.76	1 (R)	5.34	1 (I)	2.38	1 (R)	12.81
2	29.63	3 (S)	19.06	3 (S)	4.95	1 (R)	0.00	1 (R)	8.62	2 (I)	12.45
3	9.92	2 (I)	9.29	2 (I)	0.00	1 (R)	0.00	1 (R)	0.00	1 (R)	3.84
4	42.10	3 (S)	35.81	3 (S)	29.29	3 (S)	0.00	1 (R)	4.76	1 (R)	22.39
5	90.11	3 (S)	82.22	3 (S)	37.46	3 (S)	0.00	1 (R)	42.59	3 (S)	50.48
6	37.90	3 (S)	30.56	3 (S)	39.84	3 (S)	2.56	1 (R)	6.67	2 (I)	23.51
7	32.59	3 (S)	22.95	3 (S)	47.78	3 (S)	0.00	1 (R)	21.79	3 (S)	25.02
8	21.79	3 (S)	34.09	3 (S)	16.87	2 (I)	0.00	1 (R)	5.41	2 (I)	15.63
9	20.62	3 (S)	35.95	3 (S)	28.14	3 (S)	6.36	2 (I)	2.56	1 (R)	18.73
10	82.22	3 (S)	44.06	3 (S)	74.42	3 (S)	0.00	1 (R)	19.39	3 (S)	44.02
11	18.41	3 (S)	51.77	3 (S)	49.07	3 (S)	0.00	1 (R)	15.45	2 (I)	26.94
12	39.93	3 (S)	28.33	3 (S)	57.05	3 (S)	2.56	1 (R)	8.33	2 (I)	27.24
13	18.80	3 (S)	83.33	3 (S)	58.82	3 (S)	0.00	1 (R)	27.92	3 (S)	37.77
14	52.02	3 (S)	54.62	3 (S)	84.15	3 (S)	0.00	1 (R)	13.10	2 (I)	40.78
15	32.44	3 (S)	36.63	3 (S)	28.57	3 (S)	0.00	1 (R)	6.06	2 (I)	20.74
16	42.12	3 (S)	41.07	3 (S)	32.14	3 (S)	0.00	1 (R)	37.98	3 (S)	30.66
17	51.59	3 (S)	63.70	3 (S)	15.87	2 (I)	0.00	1 (R)	0.00	1 (R)	26.23
18	68.06	3 (S)	47.58	3 (S)	41.88	3 (S)	2.22	1 (R)	22.42	3 (S)	36.43
19	27.78	3 (S)	29.22	3 (S)	28.69	3 (S)	0.00	1 (R)	0.00	1 (R)	17.14
20	55.42	3 (S)	47.88	3 (S)	39.74	3 (S)	0.00	1 (R)	16.24	2 (I)	31.86
Atılır	48.99	3 (S)	86.75	3 (S)	69.87	3 (S)	7.94	2 (I)	53.59	3 (S)	53.43
Larende	63.59	3 (S)	91.07	3 (S)	74.72	3 (S)	7.72	2 (I)	37.78	3 (S)	54.98
Çumra 2001	3.33	1 (R)	3.70	1 (R)	0.00	1 (R)	0.00	1 (R)	0.00	1 (R)	1.41
Mean	39.89		43.62		37.57		1.51		15.35		27.59

Table 3. Determination of seedling reactions of 3 barley cultivars and 20 landraces to 10 *Drechslera graminea* isolates. For evaluation, a 1-3 scale developed by Tekauz (1983) was used. (R: Resistant, MR: Moderately Resistant, S: Susceptible). Numbers are mean of three replications.

Barley landraces and cultivars	Isolates										Landrace/cultivar mean
	Bilecik		Afyon		Kayseri		Sivas		Yozgat		
	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value	Mean disease percent	Scale value	
1	11.45	2 (I)	9.52	2 (I)	0.00	1 (R)	7.72	2 (I)	17.22	3 (S)	11.00
2	5.34	2 (I)	23.35	3 (S)	0.00	1 (R)	2.22	1 (R)	48.75	3 (S)	14.19
3	0.00	1 (R)	0.00	1 (R)	0.00	1 (R)	0.00	1 (R)	2.56	1 (R)	2.18
4	36.11	3 (S)	31.26	3 (S)	2.78	1 (R)	7.14	2 (I)	22.00	3 (S)	21.13
5	12.78	2 (I)	83.33	3 (S)	20.79	3 (S)	73.43	3 (S)	77.78	3 (S)	52.05
6	15.32	2 (I)	18.25	3 (S)	21.37	3 (S)	2.08	1 (R)	37.55	3 (S)	21.21
7	16.56	2 (I)	50.55	3 (S)	4.79	1 (R)	5.56	2 (I)	29.29	3 (S)	23.19
8	0.00	1 (R)	2.56	1 (R)	0.00	1 (R)	2.56	1 (R)	12.48	2 (I)	9.58
9	12.50	2 (I)	36.03	3 (S)	2.78	1 (R)	9.52	2 (I)	37.39	3 (S)	19.19
10	24.68	3 (S)	41.41	3 (S)	12.70	2 (I)	57.14	3 (S)	18.46	3 (S)	37.45
11	20.95	3 (S)	52.86	3 (S)	25.93	3 (S)	15.45	2 (I)	45.95	3 (S)	29.58
12	11.11	2 (I)	46.04	3 (S)	8.33	2 (I)	19.78	3 (S)	71.35	3 (S)	29.28
13	64.98	3 (S)	25.32	3 (S)	31.85	3 (S)	32.53	3 (S)	62.39	3 (S)	40.59
14	49.40	3 (S)	66.32	3 (S)	2.38	1 (R)	22.22	3 (S)	90.30	3 (S)	43.45
15	10.97	2 (I)	44.62	3 (S)	21.43	3 (S)	7.91	2 (I)	47.77	3 (S)	23.64
16	36.67	3 (S)	45.37	3 (S)	0.00	1 (R)	15.38	2 (I)	22.84	3 (S)	27.36
17	0.00	1 (R)	33.02	3 (S)	0.00	1 (R)	9.78	2 (I)	50.56	3 (S)	22.45
18	11.11	2 (I)	34.36	3 (S)	0.00	1 (R)	24.21	3 (S)	31.67	3 (S)	28.35
19	26.92	3 (S)	17.17	3 (S)	0.00	1 (R)	0.00	1 (R)	35.74	3 (S)	16.55
20	48.89	3 (S)	63.48	3 (S)	7.94	2 (I)	23.21	3 (S)	38.38	3 (S)	34.12
Atılır	83.01	3 (S)	77.78	3 (S)	17.78	3 (S)	25.76	3 (S)	41.71	3 (S)	51.32
Larende	54.28	3 (S)	31.31	3 (S)	25.04	3 (S)	31.94	3 (S)	82.98	3 (S)	50.04
Çumra 2001	0.00	1 (R)	0.00	1 (R)	0.00	1 (R)	4.67	1 (R)	0.00	1 (R)	1.17
General Mean	24.04		36.26		8.95		17.40		40.22		27.31

Konya (Bozkır) isolate was the most virulent isolate with a mean disease percent of 43.62. Twenty-one barley genotypes were found susceptible to Konya (Bozkır) isolate. Yozgat, Konya (Altınekin), Ankara (Akyurt) and Afyon isolates followed the Konya (Bozkır) isolate. Ankara (Haymana) isolate was the least virulent isolate with a mean disease percent of 1.51. Kayseri isolate followed this isolate with a mean disease percent of 8.95.

In addition to pathogenic variation, variation among the growth and colony characteristics were also observed in *D. graminea* cultures. Ten *D. graminea* isolates showed differences in their growth habit following ten days of growth in PDA. Isolate colors changed from whitish light gray to dark gray brown. Most of the isolates covered Petri plates following ten days of growth with the exception of Yozgat isolate. Hammouda (1988) reported variation among cultural characteristics of *Pyrenophora graminea* isolates. Mohammad and Mahmood (1976) reported morphological differences among the single spore isolates of *Helminthosporium gramineum*.

Generally 6 rowed barley varieties were reported as more susceptible to this disease than 2 rowed cultivars (Bobes et al. 1975). In our study, 4 barley landraces were 6 rowed. Barley landrace 5 was susceptible to 8 isolates, This landrace showed an intermediate reaction to Bilecik isolate and a susceptible reaction to Ankara (Haymana) isolate which was the least virulent isolate. Landrace 10 exhibited a susceptible reaction to 8 isolates. This landrace showed an intermediate reaction to Kayseri isolate and a resistant reaction to Ankara (Haymana) isolate. Landrace 17 was susceptible to 4 isolates and resistant to 4 isolates. This landrace exhibited an intermediate reaction to 2 isolates. Landrace 18 exhibited a susceptible reaction to 7 isolates. This landrace showed an intermediate reaction to Bilecik isolate and resistant reactions to Kayseri and Ankara (Haymana) isolates. Although susceptibility was common to isolates among *D. graminea* isolates, resistance was also present especially in the landrace 17.

In a study performed by Ulus and Karakaya (2007) seedling reactions of 15 barley cultivars grown in Turkey were determined under greenhouse conditions to five isolates of *Drechslera graminea.* Isolates were collected from different parts of Ankara province. Differences were observed among the reactions of the cultivars to the isolates of the fungus. There were also pathogenicity differences among the isolates. The cultivars Çumra 2001 and Yerçil 147 were resistant to all five isolates. Cultivar Sladoran was resistant to 4 isolates. The cultivars Erginel 90, Orza 96, Çetin 2000 and Aydanhanım were susceptible to three isolates of the fungus. The reactions of other varieties ranged between resistant and susceptible depending on the isolates. Isolate Dg3 was the most virulent. Also in our study cv Çumra 2001 was found resistant to all isolates.

Bayraktar and Akan (2012) reported that Turkish isolates of *Pyrenophora graminea* were homogenous. They found differences among the reactions of the Turkish barley cultivars to *P. graminea* isloates. Durusu, Balkan 96 (Igri), Çumra 2001 and Anadolu 98 cultivars were resistant to 13 isolates tested. Cultivars Atılır and Larende were susceptible to most of the isolates. In our study, cultivar Çumra 2001 was resistant to all isolates tested and cvs Atılır and Larende were susceptible to 9 isolates.

It appears that resistant genotypes exist among the Turkish barley landraces and cultivars. Resistance status of cultivars and landraces should also be tested under field conditions. Resistant cultivars and landraces could be used in breeding programs and the use of resistant cultivars and landraces by the farmers should be encouraged.

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