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RESEARCH PAPERS

Turkish barley landraces resistant to net and spot forms of *Pyrenophora teres*

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Summary. *Pyrenophora teres* is an important pathogen of barley. The pathogen has two biotypes: *Pyrenophora teres* f. *teres*, which causes the net type of net blotch, and *P. teres* f. *maculata* causing the spot type of net blotch. Turkey is an important gene centre of barley and has a rich barley landrace population. Finding disease resistant barley germplasm has potential for world agriculture. Three virulent *Pyrenophora teres* f. *maculata* (*Ptm*) isolates and three virulent *Pyrenophora teres* f. *teres* (*Ptt*) isolates were tested for their pathogenicity to 198 barley landraces, and landraces resistant to both forms of the pathogen were identified. Thirteen landraces (numbered 17, 40, 71, 98, 101, 103, 104, 143, 162, 167, 171, 183 and 185) were resistant to the *Ptm* isolates and seven (numbered 18, 21, 22, 24, 40, 71 and 153) were resistant to the *Ptt* isolates. Two landraces (40 and 71) were resistant to all six *P. teres* isolates. In addition, several of the landraces exhibited reactions to one or two isolates of *Ptt* or *Ptm*, in the resistant to moderately resistant range. Using disease resistant host genotypes will help to reduce the use of disease control chemicals, and with development of efficient host resistance strategies to combat net blotch diseases. These landraces could be used as sources of resistance for barley breeding.

Key words: *Drechslera teres*, *Hordeum vulgare*, Disease resistance.

Introduction

Barley is one of the oldest cultivated plants in the world, which has been cultivated for thousands of years (Kün, 1996). The net blotch fungus *Pyrenophora teres* (anamorph: *Drechslera teres*) belongs to the phylum Ascomycota, and has two biotypes. *Pyrenophora teres* Drechs. f. *teres* Smedeg. (*Ptt)* causes net type leaf symptoms, and *P. teres* f. *maculata* Smedeg. (*Ptm*) causes spot type symptoms on the barley leaves. These are among the most important barley diseases, which occur in many countries and causes significant economic losses (Shipton *et al*., 1973; Mathre, 1982; Karakaya *et al*., 2014). Losses due to the pathogen range between 10–40% (Mathre, 1982). Fungicide applications, cultural practices and the use of resistant cultivars are the recommended disease management methods (McLean *et al*., 2012). The prevalence of net blotch is closely related to the susceptibility of barley cultivars grown in specific areas. The most eco-friendly, practical and profitable method for net blotch control is the use of resistant barley cultivars.

Barley landraces are important sources of genetic variation (Yitbarek *et al*., 1998; Ellis *et al*., 2000). Landraces can be successfully cultivated even in unfavourable conditions, owing to their adaptability to changing environmental conditions (Allard and Bradshaw, 1964). Turkey ranks very highly regarding the abundance of landraces. Anatolian landraces and hulless barleys have been shown to be far superior to other cultivars in terms of efficiency and endurance against drought (Gökgöl, 1969). Landraces are still planted in Turkey.

Plant breeders need sustainable new resources of resistance against diseases. Efficient use of the rich genetic resources in Turkey is believed to be one of

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the best ways to combat the diseases caused by *P. teres*. Recently in Turkey, approx. 3,500 barley landraces obtained from Turkey and different parts of the world, and maintained at Anatolian Agricultural Research Institute, were renewed by the Central Research Institute for Field Crops located in Ankara, Turkey. Agromorphological, biochemical and molecular characterization of these landraces was also carried out by this Institute. Two hundred winter type landraces originating from Turkey were selected. These were obtained with single spike selection. In the study reported here, three single conidium isolates of *P. teres* f. *maculata* and three single conidium isolates of *P. teres* f. *teres*, the most virulent isolates identified in a previous study (Çelik Oğuz, 2015) were tested on these 198 landraces, to determine their resistance status against both forms of net blotch. The resistance status of these landraces to net blotch diseases has not been previously assessed.

Materials and methods

Plant material

Two hundred barley landraces were used. These were collected from various parts of Turkey and conserved by the Field Crops Central Research Institute (Ankara, Turkey). Agromorphological, biochemical and molecular characterization of these landraces was performed previously by the Field Crops Central Research Institute, and landraces suitable for winter type sowing were selected. Seed of each landrace was multiplied from a single spike. Almost all (198) of these landraces provided sufficient seeds, and these were included in the present study. Insufficient seeds were obtained from landraces Nos 43 and 116. The reactions of the landraces to virulent *Ptm* and *Ptt* isolates were determined for the first time with this study.

Pyrenophora teres isolates

In a previous study, 425 single conidium isolates of both forms of the net blotch pathogen were obtained from different regions of Turkey, and 50 isolates of *Ptm* and 40 isolates of *Ptt* were tested on a barley differential set that consisted of 25 genotypes (Wu *et al*., 2003). This determined the pathotypes of both biotypes of *P. teres* in Turkey (Çelik Oğuz, 2015). Three isolates of *Ptm* and three isolates of *Ptt* that were found to be the most virulent were used to determine seedling stage resistance of the 198 barley landraces, under controlled conditions in a greenhouse. *Ptm* isolate GPS263PTM was obtained from the Ankara-Bala region of Turkey, isolate 13-179PTM from the Kahramanmaraş-Pazarcık region, and isolate 13-167PTM from the Diyarbakır-Central region. *Ptm* isolates GPS263PTM, 13-179PTM and 13-167PTM were the most virulent *Ptm* isolates, their mean virulence values over 25 differential set genotypes (Wu *et al*., 2003) were 7.36, 7.04 and 6.84, according to the Tekauz (1985) scale (Çelik Oğuz, 2015). The response of susceptible local barley cultivar Bülbül 89 to these three isolates was, respectively, 9, 8 and 8 according to the Tekauz (1985) scale (Çelik Oğuz, 2015).

Ptt isolate GPS18PTT was obtained from the Sivas-Yıldızeli region, isolate UHK77PTT from the Kilis region, and isolate 13-130PTT from the Şanlıurfa-Ceylanpınar region. *Ptt* isolates GPS18PTT, UHK77PTT and 13-130PTT were also the most virulent *Ptt* isolates, and their mean virulence values over 25 differential set genotypes were, respectively, 5.84, 5.80 and 5.64 according to the Tekauz (1985) scale (Çelik Oğuz, 2015). The response of susceptible local barley cultivar Bülbül 89 to these isolates was, respectively, 9, 7 and 6, according to the Tekauz (1985) scale (Çelik Oğuz, 2015).

Preparation of inoculum, inoculation and incubation

Sterile mixtures of soil, sand and organic substances (60:20:20, v:v:v) were placed in plastic pots (7 cm diam.), and (depending on the quantity of available seeds of landraces) five to ten seeds were placed into each pots. The pots were maintained under greenhouse conditions before and after inoculation. Resulting plants were inoculated at growth stages 12-13 (Zadoks *et al*., 1974). Single conidia were isolated using blotter method. Diseased leaves were cut in 2–3 cm lengths, and after surface sterilization with 1% NaOCl for 1 min, they were placed into sterile Petri dishes containing wet filter paper. The Petri dishes were incubated under room conditions. Three days later, single conidia were taken using a stereomicroscope. Inoculum was prepared from cultures grown in potato dextrose agar (PDA). For inoculum production, mycelia were scraped from Petri plates using a paintbrush. Inoculum concentration was adjusted to 15–20 × 10^4 mycelial fragments mL^{-1}

(Douiyssi *et al*., 1998). One drop of Tween 20 was added to every 100 mL of inoculum (Aktaş, 1995). The temperature of the greenhouse was 18±1ºC night and 23±1ºC day with a 14h/10h light/dark regime. Following inoculation, the plants were kept covered with nylon in transparent boxes with lids for 76 h. Then, they were kept in high humidity for another 48 h after which they were uncovered and ventilated. Three replicate pots of each landrace were used in the experiment.

Evaluation of disease

After 7 d, the plants were evaluated for disease using the severity scales developed for net and spot forms of net blotch by Tekauz (1985). The scales use lesion morphology. Scale values of 1, 2 and 3 were considered as resistant. In the scale for the spot form of net blotch, seven numerical classes were recognized (1 = R: resistant, 2 = R: resistant to MR: moderately resistant, 3 = MR: moderately resistant, 5 = MR: moderately resistant to MS: moderately susceptible, 7 = MS: moderately susceptible, 8 = MS: moderately susceptible to S: susceptible, and 9 = S: susceptible). In net form scale ten numerical classes were recognized (1 = R: resistant, 2 = R: resistant to MR: moderately resistant, 3 = MR: moderately resistant, 4 = MR: moderately resistant to MS: moderately susceptible, 5 = MR: moderately resistant to MS: moderately susceptible, 6 = MR: moderately resistant to MS: moderately susceptible, 7 = MS: moderately susceptible, 8 = MS: moderately susceptible to S: susceptible, 9 = S: susceptible, and 10 = VS: very susceptible. Net blotch lesions classified as resistant or moderately resistant are small and remain restricted in size. These lesions are primarily composed of necrotic tissue, and leaf tissue surrounding each lesion appears normal green in color. Lesions classified moderately susceptible or susceptible have chlorotic surrounding zones. These zones enlarge with time and may coalesce and result in the death of entire leaves (Tekauz, 1985).

Agronomic evaluation of the barley landraces

Under field conditions, agronomic evaluations of landraces were carried out. These included: days to heading, days to maturity (d), plant height (cm), numbers of fertile heads per m^2, 1,000 kernel weights (g), grain yields (kg ha^{-1}) and cold tolerance (0–5 scale) during 2012/2013 cropping year at İkizce (Gölbaşı/Ankara) location, Turkey. These evaluations were carried out in an experiment using Augmented Experimental Design (Peterson, 1994). Planting date was 23 October, 2012, and harvesting date was 10 July, 2013. Agronomic traits were evaluated according to Ergün and Geçit (2008). Only the results of the disease resistant landraces are presented in the present paper.

Results

Three isolates of *Ptm* and three isolates of *Ptt* that were previously shown to be virulent (Çelik Oğuz, 2015) were tested on 200 barley landraces. Thirty of these landraces were six-rowed barleys and 170 2-rowed. Insufficient seeds were obtained from landraces 43 (six-rowed) and 116 (two-rowed).

Novel resistance sources to both forms of *P. teres* were identified (Table 1). Forty-eight barley landraces were moderately resistant, six were resistant-moderately resistant and one was resistant to *Ptm* isolate GPS263PTM. Seventy-four landraces were moderately resistant to the *Ptm* isolate 13-179PTM, and 74 landraces were moderately resistant, and eight landraces were resistant-moderately resistant to *Ptm* isolate 13-167PTM. Thirteen landraces were resistant to all three isolates of *Ptm* (landraces 17, 40, 71, 98, 101, 103, 104, 143, 162, 167, 171, 183 and 185). Of the resistant landraces, 23% were six-row barleys and 77% were two-rowed.

In addition, 46 landraces (landraces 1, 13, 16, 31, 41, 44, 49, 51, 54, 61, 62, 69, 85, 93, 97, 99, 100, 106, 113, 114, 115, 118, 119, 120, 121, 123, 124, 125, 126, 128, 129, 132, 136, 137, 140, 144, 145, 159, 163, 165, 172, 176, 180, 181, 182 and 187) exhibited resistance to two virulent isolates of *Ptm*, and 75 landraces showed resistance to one virulent *Ptm* isolate.

Eight landraces were moderately resistant and one was resistant-moderately resistant to *Ptt* isolate GPS18PTT. Thirteen landraces were moderately resistant and one was resistant-moderately resistant to *Ptt* isolate UHK77PTT. Sixty three landraces were moderately resistant and three were resistant-moderately resistant to *Ptt* isolate 13-130PTT. Seven landraces were resistant to all three *Ptt* isolates (landraces 18, 21, 22, 24, 40, 71, 153). Of the landraces resistant to *Ptt*, 29% were six-row barleys, and 71% were two-rowed. In addition, eight landraces (landraces 32, 79, 80, 81, 84, 127, 132, 200) were resistant to two virulent isolates of *Ptt*, and 51 landraces were resistant to one *Ptt* isolate.

Table 1. Seedling reactions on some resistant barley landraces to six virulent isolates of *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*, based on the Tekauz (1985) scale (see text). Some agronomic parameters measured for the landraces are also presented.

Landrace No	Accession No.	Row Type	Days to heading	Days to maturity (d)	Plant height (cm)	Number of fertile heads/ m^2	1,000 kernel weight (g)	Grain yield (kg ha^{-1})	Cold tolerance (0-5 scale)	*P. teres* f. *maculata* isolates			*P. teres* f. *teres* isolates		
										GPS 263 PTM	13-179 PTM	13-167 PTM	GPS 18 PTT	UHK 77 PTT	13-130 PTT
17		6-row	180	224	98	375	31,5	4220	4	**3**	**3**	**3**	4	4	5
18	ES1548-3A-0A	2-row	178	222	98	432	44,3	4481	2	7	5	5	**3**	3	**3**
21	ES1565-4A-0A	2-row	179	223	97	408	42,3	3997	3	3	5	7	**2**	2	**2**
22	ES1565-5A-0A	2-row	174	227	98	528	39,8	4466	2	7	5	5	**3**	3	**3**
24	ES1572-1A-0A	6-row	177	221	102	320	32	2231	3	7	5	5	**3**	3	**3**
40	ES2162-2A-0A	6-row	183	227	102	380	36,6	4200	3	**3**	**3**	**3**	**3**	3	**3**
71	ES2289-1A-0A	6-row	191	237	99	240	37,1	2873	3	**3**	**3**	**3**	**3**	**3**	**3**
98	ES2324-2A-0A	2-row	191	235	102	492	45,1	4966	3	**3**	**3**	**3**	6	5	5
101	ES2331-1A-0A	2-row	193	237	86	492	45,8	3972	2	**3**	**3**	**3**	4	4	5
103	ES2331-3A-0A	2-row	188	235	86	420	47,8	4355	2	**3**	**3**	**3**	6	6	5
104	ES2331-5A-0A	2-row	191	236	91	642	48,7	4840	2	**2**	**3**	**3**	6	5	5
143	ES2724-5A-0A	2-row	184	228	106	758	47,4	7426	3	**3**	**3**	**3**	5	4	4
153	ES2744-1A-0A	6-row	189	235	87	288	42,8	2071	2	3	5	5	**3**	3	3
162	ES3256-1A-0A	2-row	188	233	103	372	47,3	4404	4	**3**	**3**	**2**	6	5	5
167	ES3267-6A-0A	2-row	183	227	98	714	45,1	6620	2	**3**	**3**	**3**	6	6	6
171	ES3287-6A-0A	2-row	186	230	101	250	45,6	1880	4	**3**	**3**	**3**	6	4	4
183	ES3394-10A-0A	2-row	186	233	91	672	45,3	5423	2	**3**	**3**	**3**	6	5	5
185	ES3400-1A-0A	2-row	184	229	98	498	44,3	5188	3	**3**	**3**	**3**	6	4	4

Landraces 40 and 71 were resistant to the three *Ptm* and the three *Ptt* isolates used in this study. Both of these resistant landraces were six-row barleys. Several of the landraces exhibited resistant to moderately resistant reactions to one or two isolates of *Ptt* or *Ptm*. Three of the landraces that exhibited resistant reactions (R-MR to MR) to *Ptm* were six-row landraces and the remaining ten were two-rowed. Four of the landraces that exhibited resistant R-MR to MR reactions to *Ptt* were six-row landraces, and three were two-rowed. Resistance in Turkish six- and two-row barley germplasm has been reported previously (Karakaya and Akyol, 2006; Taşkoparan and Karakaya, 2009; Aktaşdoğan *et al*., 2013; Gerlegiz *et al*., 2014; Usta *et al*., 2014; Yazıcı *et al*., 2015).

Resistant landraces exhibited considerable variation in days to heading (174–193 d), days to maturity (221–237 d), plant height (86–106 cm), numbers of fertile heads (240–758 m^{-2}), 1,000 kernel weight (31,5–48,7 g), grain yield (1,880–7,426 kg ha^{-1}) and cold tolerance (scale values 2–4) (Table 1).

Discussion

Turkey is at the crossroads of the main barley gene centres, so this country has a rich barley landrace potential (Vavilov, 1951; Kün, 1996). Presence of genetic resources and their utilization, transfer of superior quality traits of wild relatives to cultivars via gene transfer, and reduction of the use of chemicals during crop production are important for barley (Laurei *et al*., 1992).

Frankel and Hawkes (1975) indicated the importance of plant genetic resources and emphasized the importance of wild relatives. These resources should be collected from their natural habitats and protected in stock cultures. Many resistant barley genotypes were present in centres of barley evolution areas (Afanasenko *et al*., 2000). McLean *et al*. (2009) determined resistance among the barley genotypes from Middle East.

Turkey has important barley genetic resources (Kün, 1996). Chakrabarti (1968) tested 6,246 barley varieties in the World Barley Collection for reaction to net blotch disease, and 417 varieties were found to be resistant to the disease, and 30 were highly resistant. The majority of resistant varieties were from Turkey. Khan (1969) tested 8,756 barley varieties in the World Barley Collection, which originated from Turkey, and six were highly resistant. Studies conducted in Turkey also revealed diversity of resistance and susceptibility among barley cultivars and genotypes (Karakaya and Akyol, 2006; Taşkoparan and Karakaya, 2009; Aktaşdoğan *et al*., 2013; Gerlegiz *et al*., 2014; Usta *et al*., 2014; Yazıcı *et al*., 2015).

New pathotypes of fungi can be more virulent than the established pathotypes. Resistance studies should be continous and a wide range of resistance sources should be available. There are numerous studies of the resistance of barley landraces to *P. teres*. Legge *et al*. (1996) tested 176 Turkish barley lines for reaction to *P. teres*. More resistant lines were found to the spot form of net blotch compared to the net form, and similar results occurred in the present study. Lakew *et al*. (1995) evaluated Ethiopian landraces for disease resistance and agronomic traits, and Yitbarek *et al*. (1998) evaluated Ethiopian landraces for disease resistance. Considerable variation was found among these landraces for reaction to *P. teres* and for agronomic traits, such as days to heading, days to maturity and plant height. Also in our study, considerable variation was evident among the Turkish barley landraces for disease resistance and agronomic traits. Endresen *et al*. (2011), under field conditions, evaluated 2,786 barley landraces to an isolate of *Ptt* at four different research stations during 8 years. A majority of the landraces were resistant or moderately resistant to the pathogen. In the present study, performed under greenhouse conditions, out of 198 landraces, seven were resistant (Tekauz scale ≤3) to three virulent isolates of *Ptt*.

Limited resistance to *P. teres* was found among the some landraces used in different studies. Silvar *et al*. (2010) evaluated the reactions of 159 barley landraces and 16 cultivars obtained from the Spanish Barley Core Collection to three *Ptt* isolates. The overall resistance against net blotch in the Spanish landraces was low. Most of the accessions were classified as susceptible or moderately susceptible to each of the isolates. Only one accession was resistant to all three isolates, and one was classified as moderately resistant to one isolate and resistant to two other isolates. Similarly, the cultivars also displayed low resistance levels. Neupane *et al*. (2015) tested 2,062 barley accessions obtained from the World Barley Core Collection to four *Ptm* isolates obtained from United States, Australia, New Zealand and Denmark. Only fifteen accessions were resistant to all four isolates. In Ethiopia, 900 landrace lines, from 45 populations representing three locations, tested and four lines

were resistant to net blotch (Semeane, 1995). Greater levels of resistance were found in the present study.

Jana and Bailey (1995) assessed resistance to Canadian isolates of three foliar pathogens (*Cochliobolus sativus*, *Ptt* and *Ptm*) in wild and cultivated landrace barley (*Hordeum vulgare* subsp. *spontaneum* and *H. vulgare* subsp. *vulgare*) from Turkey and Jordan. Seedlings were inoculated separately with the pathogens in growth cabinet tests. More wild than cultivated barley accessions were resistant to *C. sativus* (4.5% of wild accessions *vs.* 0.3% cultivated) and *Ptt* (21.8% *vs.* 0.5%). Equal numbers of wild and cultivated accessions were resistant to *Ptm*. A larger proportion of wild barley accessions (10.5%) had at least moderate resistance to all three leaf diseases compared to only 1.3% of cultivated accessions. The average disease rating on these accessions was less for wild barley (65%), but not significantly different from cultivated barley (73%). Resistance in wild barleys is, therefore, more common, and future studies to identify resistance should utilize more wild barley genotypes. In our study, we also observed a similar pattern related to resistance of *P. teres* biotypes, where more barley landraces showed resistant reactions to virulent *Ptm* isolates.

Several studies of host resistance to net blotch have been carried out under controlled environmental conditions. Gupta *et al*. (2003) reported that resistance to *Ptt* expressed in seedlings was frequently expressed in adult plants in the field. Similarly, Düşünceli *et al*. (2008) found a significant correlation between the seedling resistance and adult plant resistance (r = 0.53) to another important barley pathogen, *Rhynchosporium secalis*. On the other hand, Douiyssi *et al*. (1998) reported that seedling and adult plants often differed in responses to an isolate of *P. teres*. Resistant barley landraces identified in the present study as resistant to both forms of the net blotch pathogen should also be tested under field conditions to provide more reliable results.

In the present study, 13 barley landraces were found to be resistant to all three virulent isolates of *Ptm*, and seven landraces were resistant to all three virulent *Ptt* isolates. Two landraces were resistant to all six virulent isolates. In addition, several landraces exhibited resistant to moderately resistant reactions to one or two of the virulent isolates of *Ptt* or *Ptm*. More landraces were resistant to *Ptm* than to *Ptt*. This is particularly promising, since *Ptm* is more common in Turkey than *Ptt* (Karakaya *et al*., 2014).

Barley landraces are good sources of plant resistance to biotic and abiotic stresses. In order to control new pathotypes, resistance studies should be continous, and large genetic source is necessary for identification of rare resistance traits. Genetic host resistance is a desirable disease control strategy, because of environmental concerns. Disease resistant barley landraces could be used efficiently in developing disease resistant barley cultivars.

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